Summation Report of the RCI

Scientific Advisory Group

of the

Drug Testing Standards and Practices Committee

Cobalt

The Group met on April 23, 2015

Grand Hyatt, Tampa Bay, Florida, USA

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RCI Scientific Advisory Group Report: Cobalt

Members Present: Ed Martin (moderator), Dr. Adam Chambers, Dr. George Maylin, Dr. Kenneth McKeever, Dr. Mary Robinson, Dr. Scott Stanley, and Dr. Tom Tobin

Members Absent: Dr. Richard Sams

Report Compiled by Dr. Mary Robinson.

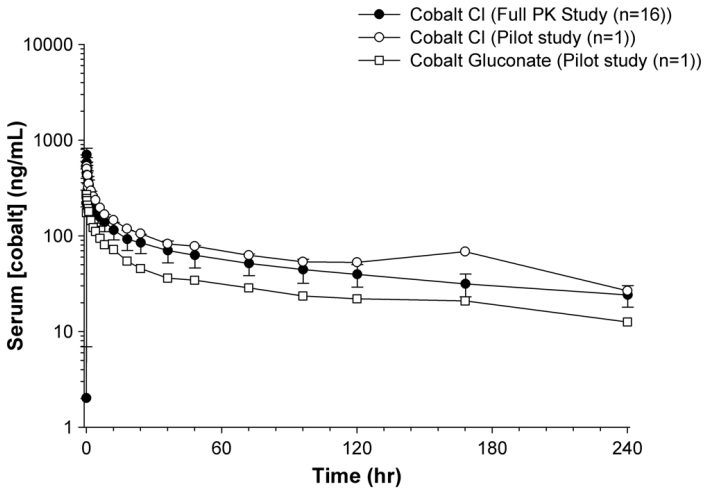
Note: Several sources of information were used to make these recommendations. The sources vary in their degree of reliability. Published peer-reviewed studies are most reliable (though not infallible) and unpublished studies are inherently less reliable since they have not undergone the peer review process. The status of the data used is included in the descriptions below in order to better inform the Committee members of the statement's reliability.

What is cobalt?

- Cobalt is a mineral that is essential in all mammals, and is normally ingested as part of Vitamin B12 (published fact).

Effects on RBC Production and Toxicity

- Administration of bulk cobalt salts to humans and other species has been demonstrated to increase red blood cell production at plasma concentrations greater than 300 ppb sustained for greater than or equal to 2 weeks, and to have toxic effects at concentrations greater than 700 ppb sustained for 8 to 40 weeks depending on the target organ (Finley, Monnot et al., 2012; Paustenbach, Tvermoes et al., 2013). Chronic oral administration was used to generate these effects.
- It is important to note that extrapolation from other species to the horse is frequently not accurate, and no published studies are available to indicate the plasma concentration that produces an effective or toxic dose in the horse. In the published study by Knych et al (Knych, Arthur et al., 2014), equine red blood cell parameters (measured at 4, 7, and 10 days post-administration) were not affected by the intravenous administration of a single dose of 49 mg/horse of cobalt, and no toxic effects were observed during the study. The maximum plasma concentration achieved in the study immediately after administration appears to be ~900 ppb (Figure 1, see below).



- Unpublished observations (RMTC report and videos; Dr. Adam Chambers) indicate that a dose of 1 to 2 g/horse of bulk cobalt salts given intravenously produces significant acute toxicity as indicated by observation of horses going down in the stall, rolling, sweating, and/or having muscle twitches for approximately 30 to 45 minutes following administration.
- It is reported that those administering bulk cobalt salts with the intent to increase red blood cell production are administering at doses to cause these acute toxic effects, creating a horse welfare concern (reported by RMTC and to Dr. Mary Robinson by practicing veterinarians in PA).
- The plasma concentration associated with these toxic effects can be assumed to be higher than those measured in the study by Knych et al (i.e. greater than 900 ppb) since no toxic effects were observed in that study. In addition, Dr. Adam Chambers shared that they have unpublished data to indicate the plasma concentration associated with these acute effects was greater than 10 ppm (i.e. > 10,000 ppb).

Race Horse Population Plasma Levels of Total Cobalt

- Population studies on total cobalt plasma concentration in the unregulated population have not been published using current technology, however, general discussion of the results of the unpublished studies indicates that the majority of horses, regardless of breed, have very low plasma concentrations of total cobalt (95% < 50 ppb in PA data set; 95% < 7 (TB and SB) or 11 (QH) ppb in RMTC data set).
    - For clarification: the PA horses included in the RMTC population analysis were part of the New Bolton Center research herd (9 TB, 5 SB) and only represent 14 of the horses in that data set (7 horses were < 0.001 ppb, 6 horses = 0.001, 1 horse = 2 ppb). The PA data set referred to above consists of 500 post-race samples (250 SB, 250 TB) that were analyzed for cobalt by Dr. Lisa Murphy's Toxicology Laboratory at New Bolton Center. Her laboratory results in a ring test were consistent with the other US toxicology laboratories (KY, CA).
    - Unpublished Canadian values for cobalt in plasma taken from Standardbred research horses (n=12) were all <1ppb.

- The population data are particularly difficult to analyze and interpret because the data are not normally distributed (left-skewed), and nothing is known about the exposure of the horses in the population to cobalt.
- Multiple differing statistical approaches have been chosen by the various research groups and there is no consensus on how to analyze these data between these groups.
- It is clear that there is a small subset of horses with very high cobalt concentrations in the population data sets, the degree of which varies significantly by region (1% > 500 ppb in PA data set from samples collected during the 2014 race season with the highest post-race value = 1,420 ppb; the maximum value in the RMTC data set was 388 ppb).

Cobalt-containing supplements and feeds

- Based on the available results of the administration of cobalt-containing supplements and feeds (most of which are also unpublished and one of which is incomplete), horses with concentrations over 50 ppb in a post-race test can be reasonably assumed to have been given cobalt salts (Unpublished data from Dr. Mary Robinson and Dr. Adam Chambers; published data from (Ho, Chan et al., 2014)).
- However, it is important to note that a study of the repeated dosing of Vita 15, the cobalt-containing product with the largest amount of cobalt, is only partially completed and the highest measured trough value to date was 46 ppb, which was in a sample taken 3 days after the 7^{th} dose of a biweekly dosing regimen as labeled on the bottle. It is possible that 24 or 48 h after that dose, the plasma concentration in that horse would have been above 50 ppb. However this also could be an outlier and will be retested to confirm the concentration is accurate. The next highest concentration achieved was 34 ppb which was after the 4^{th} dose in another horse, however the trough concentrations (the concentration measured immediately before the next sample was given) then declined to 22, 14, 15, 12, and 16 after subsequent doses (Unpublished data Dr. Mary Robinson).
- An orally administered supplement (IRON POWER) containing 44 mg of cobalt at the labeled dose was given for 14 days and resulted in a peak concentration of 22 ppb 5 min following administration and a concentration of 16 ppb 24 hours after the last dose.
- The intent of these studies is not to enable the administration of these substances. Cobalt supplementation is not medically necessary in the horse. The intent is to try to define a concentration above which it can be reasonably assumed that cobalt must have been given in the bulk salt form, which has the potential to cause the toxic effects described above. Once this is known, regulators can be assured that penalties are being assigned for an overt doping attempt, and not due to the inadvertent (or purposeful) administration of too many cobalt-containing supplements.
- As part of these studies, red blood cell parameters and equine EPO concentrations are being measured and were not observed to be affected by a single dose of the supplements tested (10% sweet feed, Red Cell, Vita 15, Vitamin B12). Red blood cell parameters are being measured as part of the repeated dosing study, but still need to be compiled and analyzed. Due to the very low dose being administered (2 ug/kg), we hypothesize that there will not be an effect,

however as stated above, extrapolation from other species is frequently not appropriate and the only way to know if there is an effect is to complete the study.

Insufficient Time for Discussion

- The Committee did not have time to discuss the work that has been done by Dr. Terrance Wan's laboratory in Hong Kong, which evaluated cobalt concentrations in urine and free cobalt in the plasma. These data have been published (Ho, Chan et al., 2014). The plasma data are not directly relevant to the USA because of differences in the methodology used by the USA testing laboratories. However, the urine data are relevant and unpublished data of a single administration of cobalt-containing supplements indicate that the proposed urine threshold by the European Horserace Scientific Liaison Committee is appropriate (Unpublished data Dr. Mary Robinson). Completion of the repeated dosing study with Vita 15 will provide additional information on the effect of the repeated supplementation of horses with cobalt on the cobalt urine concentration.

Recommendations:

1. The committee was unanimous in this recommendation.
    a. It is recommended that horses with a plasma cobalt concentration greater than 25 ppb, but less than 50 ppb be issued a warning and placed on the vet's list until the level falls below 25 ppb.
    b. Concentrations within this range may be due to the usage of cobalt-containing supplements or may be due to the administration of bulk cobalt salts as an attempt to increase red blood cell production (i.e. there is no way to confirm the type of cobalt administration that produced this result).
    c. Since cobalt supplementation is not medically necessary, levels in this range should not be allowed as it cannot be ruled out that the horse may have been receiving bulk cobalt salts.
2. The committee was unanimous in this recommendation.
    a. It is recommended that horses with plasma levels greater than 50 ppb should be penalized with a Class B penalty.
    b. The available unpublished cobalt supplement administration data suggest that values greater than 50 ppb are a result of the administration of bulk cobalt salts.
3. The committee was divided on the final recommendation (5 to 1). We were asked to consider if there was a level above which we would be comfortable with a more severe penalty.
    a. Some (5) felt that concentrations greater than 300 ppb should be severely penalized (e.g. 10 year suspension) due to evidence in other species that this concentration results in an increase in red blood cell production (Finley, Monnot et al., 2012; Paustenbach, Tvermoes et al., 2013). In addition, the risk of having a value at this level due to a cobalt containing supplement is extremely low.

b. One (1) felt the recommendation of a threshold over which severe penalties would be sanctioned is inappropriate since studies have not conclusively provided evidence of the plasma concentration needed to achieve efficacy or toxicity in the horse.

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Research article

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Pharmacokinetics and selected pharmacodynamics of cobalt following a single intravenous administration to horses

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Cobalt has been used by human athletes due to its purported performance-enhancing effects. It has been suggested that cobalt administration results in enhanced erythropoiesis, secondary to increased circulating erythropoietin (EPO) concentrations leading to improvements in athletic performance. Anecdotal reports of illicit administration of cobalt to horses for its suspected performance enhancing effects have led us to investigate the pharmacokinetics and pharmacodynamic effects of this compound when administered in horses, so as to better regulate its use. In the current study, 18 horses were administered a single intravenous dose of cobalt chloride or cobalt gluconate and serum and urine samples collected for up to 10 days post administration. Cobalt concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS) and pharmacokinetic parameters determined. Additional blood samples were collected for measurement of equine EPO concentrations as well as to assess any effects on red blood cell parameters. Horses were observed for adverse effects and heart rate monitored for the first 4 h post administration. Cobalt was characterized by a large volume of distribution (0.939 L/kg) and a prolonged gamma half-life (156.4 h). Cobalt serum concentrations were still above baseline values at 10 days post administration. A single administration of cobalt had no effect on EPO concentrations, red blood cell parameters or heart rate in any of the horses studied and no adverse effects were noted. Based on the prolonged gamma half-life and prolonged residence time, regulators should be able to detect administration of a single dose of cobalt to horses. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: cobalt; horses; detection; pharmacokinetics

Introduction

The use of cobalt as a performance-enhancing agent has been reported in human and equine athletes and stems from reports of beneficial therapeutic effects in the treatment of anaemia in patients suffering from a number of ailments, including chronic renal failure,[1–4] rheumatoid arthritis,[5] chronic suppurative infection,[6] and sickle-cell disease.[7] Cobalt acts by stabilizing a factor known as hypoxia inducible factor 1α (HIF1α). HIF1α regulates cellular and systemic oxygen homeostasis by binding to DNA coding for genes such as erythropoietin (EPO). Under normoxic conditions, HIF1α is rapidly degraded. Under hypoxic conditions, or following cobalt administration, degradation of HIF1α is inhibited, leading to activation of the EPO gene, increasing the number of reticulocytes, red blood cells and hemoglobin.[8]

While effective in the treatment of anaemia, chronic administration of cobalt, presumably due to deposition of cobalt in tissues and organs, has been associated with a number of toxic effects, which has limited its use as a therapeutic agent. Adverse effects including gastrointestinal sickness, thyroidal dysfunction, and myocardial toxicity[9] have been reported and as a result much safer agents have replaced the use of cobalt. However, even with the reported adverse effects, the use of cobalt intended as a blood doping agent persists.

It has been postulated that the enhanced erythropoiesis, secondary to increased circulating EPO concentrations, has the potential to improve anaerobic athletic performance in human athletes.[10] While cobalt is not specifically prohibited in human sports, the World Anti-Doping Agency (WADA), includes hypoxia-inducible factor stabilizers as banned substances on the 2013 Prohibited Substances List. Even so, the regulation of its use as a substance of abuse is challenging, as cobalt is a

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naturally occurring substance in the body and it is virtually impossible to differentiate between exogenous and endogenous sources. Although distinguishing exogenous cobalt from endogenous may not be possible, the large volume of distribution and the prolonged elimination half-life in humans[11–13] may prove valuable in regulating the abuse of cobalt in athletes by establishing a threshold level. To the authors' knowledge, the pharmacokinetics of cobalt in the horse have not been described and the administration of cobalt to horses as a potential performance enhancing agent, necessitates further study of this substance so as to better regulate its use. The purpose of the current study was to describe the pharmacokinetics of cobalt following intravenous administration to horses. Secondarily, because of the erythropoietic effects associated with use of cobalt in humans and anecdotal reports of adverse reactions in horses during intravenous cobalt administration we sought to describe select physiologic effects of cobalt administration to horses.

Experimental

Animals

Prior to the full pharmacokinetic study, a pilot study was conducted to evaluate potential adverse effects following intravenous cobalt administration, to determine the optimal sample collection tube type for cobalt analysis, and to select the cobalt formulation to use for the full PK study. For the pilot study, two university owned research horses, including one Thoroughbred and one Quarter Horse mare (ages: 17 and 22 years of age; weight: 552 and 634 kg) were studied. For the full PK study, 16 university-owned and exercised adult Thoroughbred horses including 8 geldings and 8 mares (age: 4 7 years; weight: 494 626 kg) were studied. Prior to and throughout the course of the study, horses were exercised five days a week. The general exercise protocol was meant to simulate the strenuous exercise of race training. The exercise regimen for these horses consists of three days per week on an Equineciser (Centaur Horse Walkers Inc., Mira Loma, CA, USA) (5 min walk; 30 min trot; 5 min walk) and two days per week on a high speed treadmill (Mustang 2200, Graber AG, Switzerland; Day 1: 5 min @1.6 m/s; 5 min @ 4 m/s; 5 min @ 7 m/s; 5 min @ 1.6 m/s all at 6% incline. Day 2: 3 min @ 1.6 m/s; 4 min @ 4.0 m/s; 2 min @ 7.0 m/s; 2 min @ 11.0 m/s and 5 min @1.6 m/s all at 3% incline). All horses were subject to regular fitness testing, including weekly heart rate measurements and calculation of V_{200} (running velocity that elicited a heart rate 200 bpm) and monthly measurements of end run plasma lactate concentrations, as a means by which to ensure that the fitness level of the horses used in this study were as comparable as possible to the average racehorse.

Before beginning the study, horses were determined healthy and free of disease by physical examination, complete blood count, and a serum biochemistry panel that included aspartate aminotransferase, creatinine phosphokinase, alkaline phosphatase, total bilirubin, sorbitol dehydrogenase, blood urea nitrogen, and creatinine. Blood analyses were performed by the Clinical Pathology Laboratory of the William R. Pritchard Veterinary Medical Teaching Hospital of the University of California, Davis, using their standard protocols. Horses did not receive any medication for at least two weeks prior to commencement of this study or any vitamin or mineral supplements for a minimum of twelve months prior to cobalt administration. Food and water were available *ad libitum* throughout the duration of the study. This study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

Instrumentation and cobalt administration

A14-gauge catheter was aseptically placed in each external jugular vein. The right jugular vein catheter was used for cobalt administration while the contralateral catheter was used for sample collection. The right jugular vein catheter was removed following dosing. For the pilot study, one horse received 169 mg of cobalt gluconate (equivalent to 22 mg of cobalt) and one horse received 109 mg of cobalt chloride (equivalent to 49 mg of cobalt). The dosing formulation was randomly assigned to each horse using a computerized random number generator. For the full PK study, all horses received cobalt chloride. As there is currently no commercially available FDA approved injectable cobalt formulation, the products used in the current study were purchased from a compounding pharmacy. The concentration of each formulation was measured as described in the Sample Analysis section below. For administration, the doses of either cobalt chloride or cobalt gluconate were diluted in 1 L of Lactated Ringers Solution and administered over 10 min via the intravenous catheter. Upon completion of administration, the catheter was flushed with heparinized saline (10 IU/mL).

Sample collection

Blood samples were collected at time 0 (prior to the start of the cobalt infusion) and at 5 and 10 min following commencement of the infusion. Additional samples were then collected at 5, 10, 15, 30, and 45 min, and 1, 2, 3, 4, 5, 6, 8, 12, 18, 24, 36, 48, 72, 96, 120, 168, and 240 h following completion of the 10 min infusion. Prior to drawing each sample of blood for analysis of cobalt concentrations, 10 mL of blood was aspirated and discarded from the catheter and T-Port extension set (combined internal volume <2 mL). The catheter was flushed with 10 mL of a dilute heparinized saline solution (10 IU/mL) following each sampling time. The jugular vein catheter, used for sample collection, was removed following the 18-h sample collection and the remaining samples collected via direct venipuncture. Blood samples were collected into serum separator tubes and placed at room temperature prior to centrifugation at 3000 rpm for 10 min at 4 °C. Serum was then immediately transferred into storage cryovials (Phenix Research Products, Chandler, NC, USA) and stored at -20 °C until analysis. For the horse receiving the cobalt chloride formulation in the pilot study, two additional sets of blood samples were collected, one set in trace metal free serum tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and a second set in trace metal free tubes containing K2EDTA (Becton Dickinson, Franklin Lakes, NJ, USA), for comparison of cobalt concentrations between different tube types. Samples were collected at 0 (immediately prior to cobalt administration) and 30 min, 4, 12, and 48 h and 5 and 7 days post cobalt chloride administration. Samples were stored as described above for the first set of samples.

Urine samples were collected at time 0 (immediately prior to cobalt administration) and at 4, 24, 48, 72, 96, 120, 168, and 240 h post cobalt administrations for the pilot and full PK study. Urine samples were collected either by free catch or urinary catheterization (mares) when necessary. Urine samples were stored at -20 °C until analysis.

Determination of cobalt concentrations

Analyses of the dosing solutions as well as cobalt concentrations for the pilot study were conducted at the California Animal Health and Food Safety Laboratory at the University of California, Davis (UCD) and analyses of samples generated from the full PK study were performed at the University of Kentucky Veterinary Diagnostic

Laboratory (UK). Quantitative methods for determining total cobalt in serum and urine by ICP-MS as described for the full PK study were based on previously published methods.[14] Complete method validation was performed prior to this study and relevant analytical figures of merit were determined. The instrument response was linear with respect to cobalt concentration over a range of 0.038 to 38.5 ng/mL. The upper limit of the linear dynamic range remains unknown; however quantitative results for all samples in the PK study were within the established range. Average recoveries and inter-assay variation coefficients were determined to be 89.2% and 1.99% for newborn calf serum overspiked at 1.000 ng/mL and 88.5% and 2.84% when overspiked at 10.00 ng/mL. Likewise, the average recovery for overspiked urine positive controls, analyzed concurrently with the study samples, was 99.8% and 4.56%. Further, the method limit of quantitation was determined to be 1.0 ng/mL. Method validation at UCD was done with reference materials NIST 1640 water with a certified value of 20.28 ng/mL and QMEQAS09B-05 blood from INSPQ (Institut national de santé publique) with a value of 4.64 ng/mL. Average recoveries and inter-assay variation coefficients were determined to be 96.9% and 9.85% for NIST 1640 and 92.7% and 8.9% for QMEQAS09B-05. Similar analytical instrumentation and operating conditions were used in both laboratories. Unless otherwise specified, all analytical instrumentation and acquisition parameters were equivalent between UCD and UK.

Ethylenediaminetetraacetic acid (EDTA in acid form; Trace Metal Grade (99.9% pure) and Triton X-100 were obtained from Sigma Aldrich (St. Louis, MO, USA). Ammonium hydroxide (Trace analysis grade), nitric acid (TraceMetal grade) and butanol (99.5%) were obtained from Thermo Fisher Scientific (Pittsburgh, PA, USA). Calibration standard solutions for analysis of the pilot samples (UCD) were prepared by diluting from single element standards of 1000 μg/mL (Inorganic Ventures; Christiansburg, VA, USA) and those for the full PK study (UK) from a custom-mixed multi-element standard solution (Inorganic Ventures) that contained 10 μg/mL cobalt. The internal standard solution was prepared from a commercially available multi-element standard solution that contained 100 μg/mL germanium (Inorganic Ventures, Christiansburg, VA, USA). Distilled, deionized water was prepared in-house using a Barnstead Mega-Pure distillation system (Model MP-6A) and a Barnstead EASYpure II RF water conditioner (Model D7031; Thermo Scientific, Dubuque, IA, USA).

Urine and serum samples were stored at -20 °C until analysis. On the day of analysis, samples were allowed to completely thaw at room temperature (20 °C to 21 °C), mixed by vortex-pulsing, and as needed, centrifuged at 3000 rpm for 10 min to pellet any undissolved particulate material in the bottom of the tube. A 200-μL aliquot of each sample (or supernatant fluid) was transferred to a labelled, 15-mL disposable centrifuge tube and diluted by the addition of 5-mL ICP-MS diluent. The ICP-MS diluent used for the pilot study analysis (UCD) consisted of 0.5% (*v/v*) nitric acid, 0.05% (*w/v*) Triton X-100, 2% (*v/v*) isopropanol and 5 ng/mL bismuth (^{209}Bi). The ICP-MS diluent used for analysis of samples generated from the full PK study (UK) was an aqueous mixture of 0.05% (*w/v*) EDTA, 1.0% (*w/v*) ammonium hydroxide, 0.05% (w/v) Triton X-100 and 2.0% (*w/v*) butanol (2.0% (*w/v*)) that contained a final concentration of 15 ng/mL germanium. Aliquots (200-μL) of calibrant solutions were also diluted with 5-mL ICP-MS diluent.

The pilot study (UCD) used NIST 1640 water, QMEQAS09B-05 blood as reference materials and equine serum from Sigma (Lot H1270) as a control. The Sigma equine serum was run in duplicate with a sample fortified at 10 ng/mL cobalt. Baseline urine from the cobalt gluconate dosed horse was used as a control and was run in duplicate with a spiked sample at 10 ng/mL cobalt. For the full PK study (UK), control samples were analyzed immediately following calibration and after every 10 to 12 samples throughout the daily sample batch. Positive controls were matrix matched or matched to the expected cobalt concentrations for the samples. Positive controls included Newborn Calf Serum (Cell Culture Grade; Sigma Aldrich, St Louis, MO, USA) fortified with 1 ng / mL cobalt and urine collected from a control horse fortified with 10 ng/mL cobalt. Negative controls were either 10% (*w/w*) nitric acid in distilled deionized water or control equine urine that was not fortified with cobalt. Aliquots (200 μL) from each of these control solutions were diluted with 5-mL ICP-MS diluent.

An Agilent 7500ce octapole reaction system inductively coupled plasma mass spectrometer (ORS)-ICP-MS; Agilent Technologies, Tokyo, Japan) operating in helium mode was used for cobalt analyses at both UCD and UK. It was equipped with a Micromist concentric glass nebulizer, a double-pass Scott-type spray chamber cooled to 2 °C, and a peristaltic pump set at 0.10 rps for sample aerosolization and introduction to the torch. The configuration of the (ORS)-ICP-MS is such that ions pass through an octapole reaction cell immediately before mass analysis in the quadrupole mass analyzer of the ICP-MS. This cell was pressurized with helium gas to minimize polyatomic interferences arising from either sample matrix components or environmental conditions that impede analysis. Operation instrumental conditions and measurement parameters are provided in Table 1. The quadrupole mass analyzer was set to perform sequential single-ion monitoring to detect the signals for *m/z* 59 and 72, corresponding to singly-charged radical cations of isotopes ^{59}Co and ^{72}Ge. In the preliminary study *m/z* 209, corresponding to ^{209}Bi was run in place of *m/z* 72.

The ratio of the detected signals for ^{59}Co and ^{72}Ge was plotted against the concentration of cobalt in the calibrant solutions to create calibration curves on a daily basis. Cobalt concentrations were interpolated from the linear trendline of the corresponding calibration curve and reported. Because the calibrant solutions, controls and samples were all diluted in the same manner, the dilution factor for the analyses was 1. Serum and urine cobalt results were reported in ng/mL. The minimum level of quantitation (MLQ) for the method was 1.0 ng/mL.

Table 1. (ORS)-ICP-MS operating conditions and measurement parameters

Parameter	Setting
RF Power	1500 W
Sample uptake rate	0.10 rps
Carrier gas flow rate	0.90 L/min
Makeup gas flow rate (Argon)	0.22 L/min
Nebulizer gas flow rate (Argon)	0.22 L/min
Signal Measurements Parameters	
Isotopes	^{59}Co and ^{72}Ge (as an internal standard)
Samples per peak	3
Sample time per point	1.5 for ^{59}Co / 0.1 for ^{72}Ge
Number of replicates	3
Reaction Cell Parameters	
Helium gas flow rate	3.4 – 4.0 mL/min (optimized daily)
Octapole bias	-18 V
Quadrupole bias	-15 V

Pharmacokinetic calculations

Compartmental analysis was used for determination of pharmacokinetic parameters for cobalt using commercially available software (Phoenix WinNonlin Version 6.0, Pharsight, Cary, NC, USA). The area under the curve and area under the moment curve were calculated using the log up-linear down trapezoidal method and extrapolated to infinity using the last measured serum concentration divided by the terminal slope λ_z.

Determination of RBC parameters

Red blood cell (RBC) parameters, including total RBC count, hemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW) were assessed prior to cobalt administration and on days 4, 7, and 10. Blood samples were collected as described for determination of cobalt concentrations into blood tubes containing EDTA. Red blood cell analyses were performed using a Siemens ADVIA® 120 Hematology System (Siemens Medical Solutions USA, Inc., Malvern, PA, USA) by the Clinical Pathology Laboratory of the William R. Pritchard Veterinary Medical Teaching Hospital of the University of California, Davis, using their standard protocols.

Determination of EPO concentrations

Samples for determination of EPO concentrations were collected in serum separator tubes as described above and stored at 4 °C. Serum EPO concentrations were measured within 24 h of collection of the final sample (10 days post cobalt administration) at the K.L. Maddy Equine Analytical Chemistry Laboratory using a commercially available equine ELISA kit (CUSABIO kit, Life Sciences Advanced Technologies, Inc., St Petersburg, FL, USA) according to the manufacturer's protocol. One hundred μL of undiluted serum from each sample was tested. Samples were run in duplicate at each time point for each horse and the average value reported. The ELISA plates were read at 450 nm with wavelength correction set to 540 nm on a Tecan Sunrise™ instrument using their Magellan™ Data Analysis Software (Tecan Trading AG, Mannedorf, Switzerland). Data were analyzed using CurveExpert Professional 1.3 (Daniel G. Hyams, Hixon, TN, USA) by generating a standard curve using a four parameter logistic (4-PL) curve fit.

Monitoring of behavioural and physiologic parameters

Horses were unrestrained for the duration of the study and were only restrained, if necessary, for sample collection. Horses were continuously monitored for any adverse or behavioral effects for 8 h post cobalt administration. Subsequent observations were made prior to blood sample collection at each time point. All assessments were made from outside the stall. Horses were equipped with a Holter monitor (Forrest Medical, East Syracuse, NY, USA) to assess any potential effect on heart rate and rhythm. Heart rate and rhythm were recorded continuously for a minimum of 30 min pre and 4 h post cobalt administration. Heart rate was determined at pre-determined time points via manual counting of P-QRS-T complexes over a 1-min time period. The percentage of atrial signals blocked by the atrio-ventricular node before and after cobalt administration was calculated using the formula, (atrial rate – ventricular rate)/atrial rate. The atrial and ventricular rates were determined by manually counting P waves and P-QRS-T complexes, respectively, over a 1-min period at pre-determined time points.

Statistical analysis

Statistical analyses, using commercially available software (SAS, Cary, NC, USA), were performed to assess significant differences in EPO concentrations, RBC parameters, heart rate and %AV block both pre and post cobalt administration for individual horses. Raw data for all variables were checked for normality using the Wilk-Shapiro test and then log transformed as necessary to bring the residual distribution in close agreement with a normal distribution. Data for all variables were subsequently analyzed using a mixed model ANOVA with repeated measures. Significance was set a $p < 0.05$.

Results

The compounded cobalt dosing solutions were tested for potency by measuring their cobalt concentrations. The calculated cobalt chloride concentration was 109 mg/mL (labelled as 200 mg/mL), based upon a measured cobalt concentration of 49 mg/mL and the calculated cobalt gluconate concentration was 1.69 mg/mL (labelled as 2 mg/mL) based upon a measured cobalt concentration of 0.22 mg/mL.

Cobalt serum concentrations were comparable between the serum and the trace metal free tubes (Table 2). Cobalt concentrations

Table 2. Cobalt concentrations in whole blood and serum following intravenous administration of 109 mg of cobalt chloride to one horse in the pilot study. The limit of quantitation of the analytical method was 1.0 ng/mL

Time	Whole Blood Concentration (ng/mL)	Serum Concentration (ng/mL)	Serum Concentration (trace element free tubes) (ng/mL)
Baseline	< 1.0	< 1.0	< 1.0
30 minutes	305	429	431
4 hours	164	236	237
12 hours	106	146	148
48 hours	46	78	80
5 days	25	53	61
7 days	23	68	NS

NS, no sample collected.

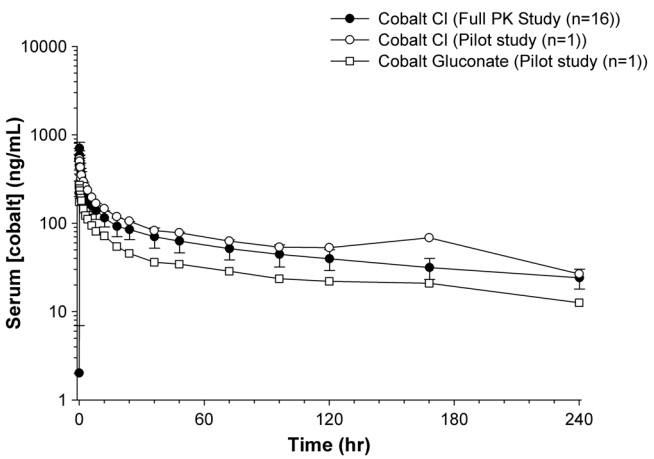


Figure 1. Serum cobalt concentration versus time curve following intravenous administration of 109 mg of cobalt chloride (49 mg of cobalt) or 169 mg of cobalt gluconate (22 mg of cobalt) to horses.

were 1.4–3.0 times higher in serum as compared to whole blood at the time points selected for measurement (Table 2). Cobalt serum concentration versus time curves for the pilot and full PK studies are depicted in Figure 1. Cobalt was detected in all pre-administration samples but the average concentration was below the LOQ of 1 ng/mL in serum. Cobalt concentrations remained above baseline values at 10 days post administration (the last time point sampled). Based on coefficient of variation, Akaike Information Criterion[15] and visual inspection of the residual plots, a three-compartment model infusion model ($C_p = Ae^{-\alpha t} - e^{-\alpha t^*} + Be^{-\beta}$

Table 3. Pharmacokinetic parameters of cobalt following a single intravenous administration of 49 mg of cobalt (109 mg cobalt chloride) or 22 mg cobalt (169 mg cobalt gluconate) to 2 sedentary research horses. All values in this table were generated using compartmental analysis

	$AUC_{0\text{-inf}}$ (h*μg/mL)	AUMC (h*h*μg/mL)	MRT (h)	Vdss (L/kg)	V_1 (L/kg)	V_2 (L/kg)	V_3 (L/kg)	Alpha HL (h)	Beta HL (h)	Gamma HL (h)	Cl (mL/min/kg)
Co chloride	21.9	3984	182	0.737	0.099	0.124	0.514	0.143	4.45	137	0.068
Co gluconate	9.79	1841	188	0.667	0.118	0.086	0.426	0.536	6.43	147	0.059

Table 4. Pharmacokinetic parameters of cobalt following a single intravenous administration of 49 mg of cobalt as cobalt chloride to 16 exercised Thoroughbred horses. All values in this table were generated using compartmental analysis.

	$AUC_{0\text{-inf}}$ (h*μg/mL)	AUMC (h*h*μg/mL)	MRT (h)	Vdss (L/kg)	V_1 (L/kg)	V_2 (L/kg)	V_3 (L/kg)	Alpha HL (h)	Beta HL (h)	Gamma HL (h)	Cl (mL/min/kg)
Horse 1	14.0	2567	184	1.22	0.176	0.232	0.809	0.630	6.45	141	0.111
Horse 2	21.0	3350	159	0.679	0.130	0.074	0.475	0.640	4.02	120	0.071
Horse 3	21.0	5240	249	1.12	0.162	0.246	0.709	1.16	13.9	200	0.075
Horse 4	16.5	3023	184	1.00	0.125	0.130	0.741	0.788	6.12	145	0.091
Horse 5	15.7	2516	160	0.80	0.128	0.143	0.524	0.951	8.11	128	0.083
Horse 6	21.6	4598	213	0.922	0.169	0.161	0.592	0.679	6.87	161	0.072
Horse 7	17.6	3917	223	1.10	0.155	0.165	0.784	1.14	8.56	176	0.083
Horse 8	44.7	14341	321	0.713	0.148	0.193	0.372	1.66	23.3	253	0.037
Horse 9	31.5	12016	382	1.13	0.168	0.305	0.653	1.68	25.8	306	0.049
Horse 10	17.6	2733	156	0.806	0.157	0.151	0.500	0.981	8.49	123	0.086
Horse 11	19.1	2731	143	0.731	0.053	0.015	0.528	0.021	2.49	106	0.085
Horse 12	17.5	2666	152	0.839	0.044	0.114	0.682	0.022	3.00	117	0.092
Horse 13	13.9	2031	146	0.938	0.133	0.198	0.607	0.418	6.17	114	0.106
Horse 14	14.3	2107	148	0.926	0.084	0.143	0.699	0.082	2.68	111	0.104
Horse 15	23.3	4548	195	0.833	0.125	0.139	0.568	0.393	5.50	148	0.071
Horse 16	13.3	2465	185	1.28	0.116	0.188	0.978	0.347	5.72	148	0.116
Mean	20.2	4428	200	0.939	0.129	0.162	0.639	0.72¥	8.63¥	156¥	0.083
Median	17.6	2878	184	0.924	0.131	0.156	0.630	0.66	6.52	146	0.084

$AUC_{0\text{-inf}}$, area under the plasma concentration time curve from 0 to infinity; AUMC, area under the moment curve; MRT, mean residence time; Vd_{ss}, volume of distribution at steady state; Cl, clearance. ¥ harmonic mean

Table 5. Urine cobalt concentrations following intravenous administration of 49 mg of cobalt as cobalt chloride or 22 mg cobalt as cobalt gluconate to horses

	Pilot Study		Full Study	
Time (hr)	Co Chloride (n = 1)	Co Gluconate (n = 1)	Co Chloride (n = 16)	
	(ng/mL)	(ng/mL)	Mean (±SD) (ng/mL)	Median (ng/mL)
Baseline	< 1	< 1	2.3 ± 1.2	2
4	7687	3281	3855 ± 1378	3511
24	730	498	240 ± 69	222
48	295	108	91 ± 37	87
72	221	67	48 ± 21	53
96	125	41	34 ± 10	29
120	90.0	30	29 ± 8	29
168	50	47	18 ± 5	20
240	NS	NS	14 ± 5	13

NS, no sample collected.

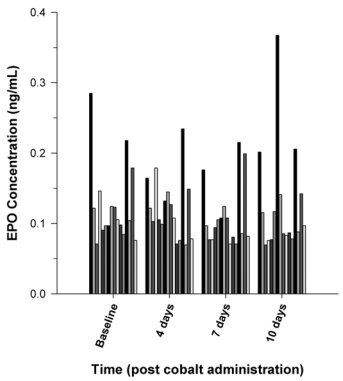
Table 6. Red blood cell parameters following intravenous administration of 49 mg of cobalt (109 mg cobalt chloride) to 16 horses

	RBC (M/μL)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)
Baseline	8.6 ± 0.5	14.2 ± 0.9	40.1 ± 2.3	46.8 ± 1.3	16.6 ± 0.4	35.4 ± 0.6	16.7 ± 0.5
Day 4	8.4 ± 0.5	13.9 ± 0.8	40.5 ± 2.2	48.5 ± 1.4	16.6 ± 0.5	34.2 ± 0.7	19.0 ± 1.0
Day 7	8.2 ± 0.5	13.6 ± 0.8	38.5 ± 2.1	46.9 ± 1.4	16.6 ± 0.6	35.4 ± 0.8	16.6 ± 0.3
Day 10	8.1 ± 0.5	13.5 ± 0.9	38.6 ± 2.4	47.8 ± 1.8	16.7 ± 0.5	34.9 ± 1.0	18.1 ± 1.4

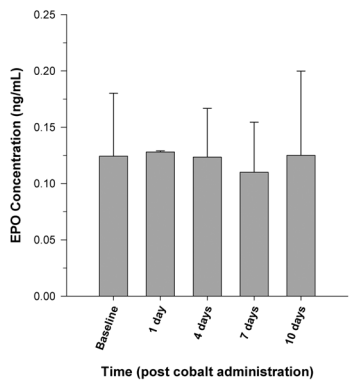
RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width.

^{t}- $e^{-\beta t^*}$ + C^{-Yt} - e^{-Yt^*}) with a weighting factor of 1/ gave the best fit to cobalt concentration data points from individual animals. Pharmacokinetic modeling was based on the measured cobalt concentrations. Selected pharmacokinetic parameters are listed in Tables 3 and 4 for the pilot and full studies, respectively. The volume of distribution was large and cobalt demonstrated a prolonged gamma half-life. Cobalt urine concentrations are reported in Table 5 for both studies. Urine cobalt in pre-administration samples averaged 2.3 ± 1.2 ng/mL in the 16 exercised horses.

No adverse reactions or behavioral effects were noted at any time post cobalt administration. There were no significant differences noted in red blood cell parameters (Table 6) or EPO concentrations (Figure 2) at any of the time points assessed following cobalt administration. Changes in heart rate ranged from -6.7% (decrease from baseline) to +6.8% (increase from baseline). The %AV block, relative to baseline, ranged from 1.6 to 7.8%. Changes in heart rate and % AV block, were not significantly different from baseline at any time post cobalt administration.



A. Erythropoeitin (EPO) concentrations in individual horses following a single intravenous administration of 109 mg/mL (49 mg of cobalt) to 16 exercised Thoroughbred horses.



B. Average erythropoeitin (EPO) concentrations following a single intravenous administration of 109 mg/mL (49 mg of cobalt) to 16 exercised Thoroughbred horses.

Figure 2. (A) Erythropoeitin (EPO) concentrations in individual horses following a single intravenous administration of 109 mg/mL (49 mg of cobalt) to 16 exercised Thoroughbred horses. (B) Average erythropoeitin (EPO) concentrations following a single intravenous administration of 109 mg/mL (49 mg of cobalt) to 16 exercised Thoroughbred horses.

Discussion

Anecdotal reports of illicit administration of cobalt salts at doses in excess of 200 mg to performance horses, for its suspected performance enhancing effects has led us to investigate the pharmacokinetics and pharmacodynamic effects of cobalt chloride and cobalt gluconate when administered to this species. A pilot study was conducted initially due to anecdotal reports of toxicity following intravenous cobalt administration. For the pilot study, two commonly used formulations of cobalt (cobalt gluconate and cobalt chloride) were administered, and although only one horse was studied per formulation, the pharmacokinetic parameters were comparable between the two. Cobalt chloride was chosen for the full study because it was readily available. It is important to note that as there is no Food and Drug Administration approved injectable product, cobalt formulations were purchased from a compounding pharmacy for use in the current study. As such, the actual concentration of the product was measured to ensure that it was the same as described on the label. In this case, both cobalt gluconate and cobalt chloride concentrations in the purchased products were much less than the labelled concentration. However, while concentrations were lower in the current study this may not always be the case. The potential for higher than labelled concentrations, raises concerns with respect to potential dose dependent adverse effects, associated with the higher concentrations.

Following injection of radiolabelled cobalt chloride to laboratory animals, cobalt reportedly concentrates in the liver, kidneys, skeleton and skeletal muscle. Hollins and McCullough[16] reported that at 10 72 h post intraperitoneal administration of radiolabelled cobalt chloride, the liver, skeleton and muscle contained 20 25% of total body activity with 7 8% accumulating in the kidney. In the same study, at 386 days post administration, 65% of the total body activity was localized in the skeleton and 7% in the liver. In mice, cobalt disappearance from blood was nearly complete 24 h after injection of cobalt chloride.[17] Interestingly, at 24 h onwards, large concentrations of cobalt were found in cartilage of the trachea and larynx and bones of the skull, the periosteum of the vertebrae and the pelvic bone.[17] While it was not possible to determine the distribution pattern of cobalt in the current study, based on the large volume of distribution (Vd_{ss}: 0.93 L/kg), cobalt also appears to be widely distributed in horses. This is similar to previous reports in humans, whereby the Vd_{ss}, was reported to be 0.6 L/kg following intravenous administration of radiolabelled cobalt chloride.[13] In that same study, the investigators hypothesized, based on a whole body scan, that the large Vd_{ss} was due to accumulation of cobalt (50% of the dose) in the liver.[13]

In addition to concentrating in a number of organs, cobalt is capable of partitioning into red blood cells (RBC). Partitioning of cobalt into RBCs has been attributed to calcium pumps and uptake appears to be irreversible due to binding of cobalt to cytosolic components.[18] While cobalt can reach high concentrations in RBCs relative to serum, in rats this occurs only after long-term exposure.[19] Following short-term exposure, the majority of administered cobalt is found in serum, with concentrations 1 2-fold higher than that in RBCs.[12,20] This is in close agreement with the results of serum and whole blood analysis in the current study, whereby concentrations of cobalt in whole blood were lower than that of serum at the time points selected for testing (Table 2; baseline to 7 days post administration) following a single administration (short-term exposure). However, it is important to note that this profile may change following chronic administration of cobalt to horses.

Of particular importance when regulating the use of cobalt in performance horses is that cobalt administration appears to be associated with long-term retention, which may aid in detecting illicit administration. In the presently reported study, the gamma half-life was prolonged following intravenous administration (4.4 to 10.5 days); however, it is important to note that serum concentrations were above the pre-dose concentration were still easily quantifiable in the last sample collected. The elimination half-life of inorganic cobalt in humans varies greatly from study to study and appears to be dependent upon the duration of sample collection.[21] Reports of very prolonged biological half-lives for cobalt in humans are common.[11,12,22,23] In one study, following a single intravenous dose to humans, 40% of the administered cobalt was excreted during the first 24 h post administration and 70% within one week.[12] In that same study, 10% of the administered dose was still present one-year post cobalt administration.[12]

The effectiveness of cobalt in increasing RBC production has been demonstrated in humans.[1–7] In the current study, there was no significant change in EPO concentrations following cobalt administration over the 10-day study period. It should be noted, however, that only a single cobalt administration was studied and the results may be different with multiple or chronic administration. Even if cobalt is ultimately proven to increase RBC production in horses, extrapolation from one species, especially human to horse, should be done with extreme caution. Unlike humans, horses, because of their contractile spleen, are capable of haemoconcentration. With respect to fit racehorses, haematocrit can easily reach up to 65% when running at VO_2 max. If cobalt does in fact increase RBCs in horses, administration to a racehorse that is already reaching a haematocrit of 65% can increase the potential for adverse cardiovascular complications.

In summary, this study described plasma and urine cobalt concentrations following intravenous administration of cobalt chloride and cobalt gluconate. The rapid rise in cobalt concentrations over baseline levels, the prolonged retention time and subsequent long gamma half-life suggest that detection of cobalt administration may be possible for several days and possibly weeks following administration of a single dose. Although EPO concentrations did not change in the current study and no adverse effects were noted, further study may be necessary to determine if this occurs with long-term exposure.

Acknowledgements

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REVIEW ARTICLE

A review of the health hazards posed by cobalt

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Abstract

Cobalt (Co) is an essential element with ubiquitous dietary exposure and possible incremental exposure due to dietary supplements, occupation and medical devices. Adverse health effects, such as cardiomyopathy and vision or hearing impairment, were reported at peak blood Co concentrations typically over 700 μg/L (8–40 weeks), while reversible hypothyroidism and polycythemia were reported in humans at ~300 μg/L and higher (≥2 weeks). Lung cancer risks associated with certain inhalation exposures have not been observed following Co ingestion and Co alloy implants. The mode of action for systemic toxicity relates directly to free Co(II) ion interactions with various receptors, ion channels and biomolecules resulting in generally reversible effects. Certain dose–response anomalies for Co toxicity likely relate to rare disease states known to reduce systemic Co(II)-ion binding to blood proteins. Based on the available information, most people with clearly elevated serum Co, like supplement users and hip implant patients, have >90% of Co as albumin-bound, with considerable excess binding capacity to sequester Co(II) ions. This paper reviews the scientific literature regarding the chemistry, pharmacokinetics and systemic toxicology of Co, and the likely role of free Co(II) ions to explain dose–response relationships. Based on currently available data, it might be useful to monitor implant patients for signs of hypothyroidism and polycythemia starting at blood or serum Co concentrations above 100 μg/L. This concentration is derived by applying an uncertainty factor of 3 to the 300 μg/L point of departure and this should adequately account for the fact that persons in the various studies were exposed for less than one year. A higher uncertainty factor could be warranted but Co has a relatively fast elimination, and many of the populations studied were of children and those with kidney problems. Closer follow-up of patients who also exhibit chronic disease states leading to clinically important hypoalbuminemia and/or severe ischemia modified albumin (IMA) elevations should be considered.

Keywords

Absorption, cobalt, medical devices, regulatory guidelines, toxicology

History

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Table of Contents

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Introduction

Cobalt (Co) is a component of cyanocobalamin, an essential vitamin (vitamin B_{12}) that is required for the production of red blood cells (RBCs) and the prevention of pernicious anemia (Barceloux, 1999). Due to its ability to stimulate hemoglobin and RBC production, Co was historically used to treat certain types of anemia (Stokinger, 1962). Typical adult doses ranged from 25 to 150 mg $CoCl_2$/d (commonly administered as enteric-coated tablets), although doses as high as 300 mg $CoCl_2$/d have reportedly been used (Gardner, 1953; Rohn & Bond, 1953; Rohn et al., 1953; Taylor et al., 1977; Wolf & Levy, 1954). The therapeutic use of Co to treat anemia was associated with occasional side effects: primarily thyroid dysfunction in children and, to a lesser degree, reversible vision and hearing impairment in adults (Bowie & Hurley, 1975; Duckham & Lee, 1976; Gross et al., 1955; Kriss et al., 1955; Licht et al., 1972; Sederholm et al., 1968). Certain individuals, specifically sickle cell children and adult patients with renal failure, developed these symptoms at lower Co doses than other patients on similar Co therapies (Berk et al., 1949; Bowie & Hurley, 1975; Duckham & Lee, 1976; Gross et al., 1955; Jaimet & Thode, 1955; Kriss et al., 1955; Robinson et al., 1949; Tevetoglu, 1956). The use of Co for treating anemia generally ended by the 1970s, when more efficacious drugs became available.

Co was also used as a foam stabilizer in beer in the 1960s. Unfortunately, this practice led to the development of cardiomyopathy in some heavy beer drinkers (e.g., ~15–30 beers per day). Interestingly, Kesteloot et al. (1968) found that well-nourished beer drinkers experienced no cardiomyopathic effects at an estimated dose of 0.09 mg Co/kg-d, while malnourished beer drinkers with the identical estimated Co dose (0.09 mg Co/kg-d) suffered severe cardiomyopathic effects, and often death (Kesteloot et al., 1968). When considered in the context of the increased susceptibility to thyroid and neurological responses in sickle cell and dialysis patients, there is historical evidence suggesting that certain disease states may render individuals more susceptible to Co toxicity (Berk et al., 1949; Gross et al., 1955; Jaimet & Thode, 1955; Keitel, 1955; Kriss et al., 1955; Robinson et al., 1949; Schleisner, 1956; Tevetoglu, 1956).

While the use of Co to treat anemia or as a beer foam stabilizer ended decades ago, several subpopulations with elevated Co exposures are known to currently exist. For example, consuming dietary supplements has become increasingly popular, and numerous Co-containing supplements are now available for sale in the United States (US). Concerns have been raised regarding the potential misuse of Co as a blood doping agent by athletes (Jelkmann & Lundby, 2011; Lippi et al., 2006). Although uncommon, daily doses of $CoCl_2$ averaging between 0.5 mg Co/d up to 1.12 mg Co/d have been recommended by some homeopathic doctors to correct hyperexcretion of estrogen that sometimes occurs during female hormone replacement therapy (Wright et al., 2005).

Most recently, concerns have been raised about elevated blood Co concentrations in patients with Co-containing hip implants (Ebreo et al., 2011; Hasegawa et al., 2012; Macnair et al., 2012). At the same time, our general understanding of the dose–response relationships between Co exposure and adverse health effects has progressed substantially over the past few years. This allows us to better characterize the possible health risks of exposed populations. For example, the EPA has issued a provisional peer reviewed toxicity value (oral reference dose) for Co (USEPA, 2008), Finley et al. (2012b) have proposed a chronic oral reference dose for Co, and Unice et al. (2012) have published a biokinetic model that can be used to estimate Co whole blood concentrations as a function of oral Co dose. Finley et al. (2012a) used this model to estimate blood Co concentrations at which health effects are expected to occur in humans and animals. Tvermoes et al. (2013b) recently reported the findings of a human study in which healthy adult male volunteers ingested approximately 0.4 mg Co/d as $CoCl_2$ in a liquid dietary supplement for 15 or 16 d.

Our analysis indicates that the questions regarding potentially susceptible populations may be answered by a conceptual model that takes into account the equilibrium between free and protein-bound Co in the blood compartment. In healthy individuals, approximately 90%–95% of blood Co is bound to serum albumin (Jansen et al., 1996), and much of the remainder is likely to be bound to smaller sulfhydryl biomolecules such as lipoic acid and glutathione; this bound fraction is in equilibrium with the free (unbound) ionic Co(II) fraction. Unlike bound Co, free ionic Co(II) can exert toxic effects by interacting with a complex array of biological receptors and proteins to stimulate erythropoiesis, foster Fenton-like generation of reactive oxygen species, interfere with mitochondrial function, inhibit thyroidal iodine uptake, and alter calcium (Ca^{2+}) homeostasis (Bucher et al., 1990; Karovic et al., 2007; Mao et al., 1996; Sederholm et al., 1968; Shrivastava et al., 2010; Weakly, 1973). Hence, theoretically, significant changes in homeostatic mechanisms that shift the total blood Co equilibrium towards increasing levels of free Co(II) ions could render an individual susceptible to the adverse effects at blood Co concentrations that would normally not pose a risk to a healthy individual.

The purpose of this paper is to review the chemistry, pharmacokinetics and toxicology of Co. We propose a unifying theory which attempts to explain why certain populations are more susceptible to the potential adverse effects of Co. Since numerous animal and human toxicology studies have been recently published, and because the most recent regulatory toxicology review (ATSDR, 2004) was released almost 10 years ago, we provide an updated review of the relevant acute, subchronic, and chronic toxicology and epidemiology studies involving exposure to Co. We evaluate the role of free Co(II) ions in the proposed molecular mechanisms of Co toxicity and the importance of free Co(II) ions in understanding how Co contributes to the etiology of systemic health effects, such as hypothyroidism, polycythemia, cardiomyopathy and neuropathies. Further, we discuss subpopulations that are currently known to be experiencing elevated Co exposures. Recently conducted human studies that involved ingesting an over-the-counter liquid Co dietary supplement for approximately 2 weeks, 1 month and 90 days are also described (Paustenbach et al., 2013; Tvermoes et al., 2013a,b).

Background

Occurrence, sources and environmental fate

Co is a prominent component of certain ores in conjunction with nickel (Ni), silver (Ag), lead (Pb), copper (Cu) and iron (Fe): linnaeite (Co_3S_4), carrolite ($CuCo_2S_4$), safflorite ($CoAs_2$), skutterudite ($CoAs_3$), erythrite ($Co_3(AsO_4)$-$8H_2O$) and glaucodot (CoAsS) (IARC, 1991; Lison, 2007; Merian, 1985; Smith & Carson, 1981). The largest reserves of Co are located in the Congo, Zambia, Australia, New Caledonia, Cuba and Russia. Current Co production in the US is predominantly from scrap metal recycling, with relatively minor amounts produced as a byproduct of mining for other metals (USGS, 2011).

Drinking water infrequently contains Co at concentrations of 0.1–5 μg/L, with surface waters and coastal seawater more commonly containing detectable levels because of transport of dissolved Co that is bound to natural organic substances (Friberg, 1977; Hamilton, 1994; Lison, 2007). Co content in US soils is reported to be on average 8.3 μg/kg (Schroeder et al., 1967), with a variation generally depending on the presence of certain types of Co-rich rocks (mafic and ultramafic igneous and mantle-type) that often contain 40–100 mg/kg (Hamilton, 1994).

While atmospheric deposition and precipitation can lead to an accumulation of Co compounds in surface waters, soils and sediments, their ultimate fate depends on sorption and solubility properties that can vary greatly by compound. Less soluble Co compounds and Co metal may settle in the environment or be sorbed directly into sediments (Albrecht, 2003), but environmental conditions favoring release of Co(II) ions may lead to complexation with soluble organics substances, which can lead to further migration (Burba et al., 1994; Jackman et al., 2001; Zhang et al., 1990). Local environment, pH, Eh, the presence and concentration of anions (Cl^-, OH^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}), and dissolved organic matter content can greatly modify free Co ion movement. For example, lower pH is thought to reduce Co-binding to particulate matter, and increasing Eh increases dissolved Co concentrations in water. Mantoura et al. (1978) modeled equilibrium states of divalent Co species in fresh water, and based on the modeled equilibrium concentrations, the substances are ranked in the following order: free $Co^{+2} > CoCO_3 > CoHCO_3^+ >> CoSO_4 >$ Co-humic acid. Tipping et al. (1998) estimated that equilibrium conditions in freshwater would be comprised of about 70% carbonate complexes, and ~25% as free divalent Co. Thus, while most metals in sediments, soils and water are transported primarily as suspended solids, some Co compounds may be transported substantially by environmental conversion to free ionic and/or complexed soluble forms (Smith & Carson, 1981).

Cobalt products and uses

Co has been used for thousands of years to color glass, pottery and jewelry a rich blue (IARC, 1991). The Babylonians and Egyptians used it extensively, and it has been used as a blue glaze for Danish porcelain since 1888 (Christensen & Poulsen, 1994). Leonardo DaVinci has been credited as one of the first artists to use Co in oil paints (Barceloux, 1999). However, it was not until the eighteenth century that Co was successfully isolated and identified as an element (Barceloux, 1999). The main use of Co remained as a coloring agent until the twentieth century, when its use in industrial applications began. In 1923, the discovery that Co mixed with tungsten carbide produced ''hard metal'' initiated its use in a variety of industrial applications, and, in 1933, Co was used as a constituent in the first permanent magnetic alloy (Barceloux, 1999; WHO, 2006).

Co metal has specific properties that make it suitable for a wide variety of industrial applications, including excellent corrosion resistance and magnetic conductivity (Table 1). When alloyed with other metals, such as tungsten or Cr, high temperature resistance, hardness, and good wear characteristics are observed (Cobalt Development Institute (CDI), 2012; Jensen & Tuchsen, 1990). As such, the primary uses of Co metal are: (1) high temperature, corrosion-resistant superalloys (e.g., used in turbine aircraft engines); (2) magnetic alloys with aluminum (Al), Cu, Ni or titanium (Ti); (3) high-strength steels; (4) electro-deposited alloys; (5) as a component of lithium ion batteries and Ni/Cd or Ni-metal hydride batteries; and (6) as a binding agent for metal carbides (USGS, 2011). Co compounds are also used as pigments in glass, ceramics and paints, as catalysts in petroleum refining, as paint driers, and as trace element additives in agriculture and medicine (ATSDR, 2004; Barceloux, 1999; IARC, 2006; USGS, 2011). According to data from 2010, the major uses of Co in the US include: superalloys (49%), chemical applications (29%), metallic applications (15%), and cemented carbides for cutting (7%) (USGS, 2011).

Physical and chemical properties

Co is among the first transition metals of Group VIII of the Periodic Table, along with Fe and Ni (Barceloux, 1999; Leonard & Lauwerys, 1990; Venugopal & Luckey, 1978). The occurrence of these three metals in the same chemical group and their similar ionic radius (0.77, 0.72 and 0.69 Å, respectively) is of toxicological importance because the smaller but

Table 1. Solubility and industrial uses of cobalt and cobalt compounds (Barceloux, 1999; Jensen & Tuchsen, 1990; Lison, 2007).

Compound name	Molecular formula	M.W.	CAS number	Industrial uses	Solubility	
					In water (temperature)	In serum (temperature)
Cobalt metal	Co	58.9	7440-48-4		Poorly soluble*	200 mg/L (37 °C)
Cobalt(II) oxide	CoO	74.9	1307-96-6	Chemicals, catalyst, pigments	Poorly soluble; 3.13 mg/L	273 mg/L (37 °C)
Cobalt(III) oxide	Co_2O_3	165.9	1308-04-09		Poorly soluble	
Cobalt(III) oxide hydrate	$Co_2O_3 \cdot H_20$	183.9	12016-80-7		Poorly soluble; 0.84 mg/L (37 °C)	53.9 mg/L (37 °C)
Cobalt(II, III) oxide	Co_3O_4	240.8	1308-06-1	Enamels, semiconductors	Poorly soluble	
Cobalt(II) hydroxide	$Co(OH)_2$	93.0	21041-93-0	Paints, chemicals, catalysts, printing inks	Poorly soluble; 3.2 mg/L (18 °C)	
Cobalt(III) hydroxide	$Co(OH)_3$	110.0	1307-86-4		Poorly soluble; 3.2 mg/L (20° C)	
Cobalt(II) sulfide	CoS	91.0	1317-42-6	Catalyst	Poorly soluble; 3.8 mg/L (18 °C)	
Cobalt(II) carbonate	$CoCO_3$	118.9	513-79-1	Pigments, ceramics, feed supplements, catalyst	Soluble; 1.1 g/L (15 °C)	
Cobalt(II) nitrate hexahydrate	$Co(NO_3)_2 \cdot 6H_20$	291.0	10026-22-9	Pigments, ceramics, feed supplements, catalyst, chemicals	Soluble; 134 g/L (0 °C)	
Cobalt(II) acetate	$Co(CH_3COO)_2$	177.0	71-48-7	Driers for lacquers and varnishes, sympathetic inks, catalysts, pigment for oil-cloth, mineral supplement, anodizer, stabilizer for malt beverages	Soluble; 380 g/L (25 °C)	
Cobalt(II) sulfate	$CoSO_4$	155.0	10124-43-3	Foam stabilizer in malt beverages	Soluble; 393 g/L (25 °C)	362 g/L (20 °C)
Cobalt(II) chloride	$CoCl_2$	129.8	7646-79-9	Foam stabilizer in malt beverages, mineral supplement	Soluble; 529 g/L (20 °C)	

*Solubility <0.1 g/L; no exact numerical values available.

chemically similar Co and Ni ions can occupy the same binding sites as divalent Fe ions (Maxwell & Salnikow, 2004). As discussed later in this review, ionic Co and Ni may substitute for ionic Fe and Zn in various biomolecules (e.g., phthalate dioxygenase, scaffolding proteins required for the biosynthesis of Fe–S clusters, and DNA repair proteins) that modulate the fate and transport of divalent metals within the body. These biomolecules are recognized to have unique interactions with various body feedback systems that regulate Fe and oxygen homeostasis (Ke & Costa, 2006; Maxwell & Salnikow, 2004).

Pure Co is a steel-gray, shiny, hard metal and has a molecular weight of 58.9 g/mol. Co metal that is commonly used in various metal alloys is in the ground state (zero valence state), and Co compounds occur in two predominant oxidation states (+2 and +3). While Co(II) and Co(III) are the common oxidation states of Co, Co(II) is far more stable in normal aqueous conditions; Co(III) may be stabilized by changing the ligand environment (Krupka & Serne, 2002). Co is present in its +2 valence state in most commercially available Co compounds and in the environment. Currently available data indicate that implant derived Co from Co-containing metal prosthesis exists primarily in the Co^{2+} oxidation state (Goode et al., 2012; Hart et al., 2010). This is the same form of Co (Co^{2+}) found in Co dietary supplements. While Co^{3+} is thermodynamically unstable under typical redox and pH conditions, the interconversion between $Co^{2+} \leftrightarrow Co^{3+}$ is important in the biological reactions of vitamin B_{12} (Gal et al., 2008; Lauwerys & Lison, 1994; Leonard & Lauwerys, 1990; TPMC, 2002).

Water-soluble Co compounds release Co(II) ions into solution, which, in turn can form various complexes with organic or inorganic anions, with equilibrium conditions depending on Eh, pH and the presence of anions (Smith & Carson, 1981). An example Eh-pH diagram for Co in fresh water systems is shown in Figure 1. In general, greater acidity leads to higher free Co(II) concentrations in solution, and greater alkalinity leads to the formation of Co–carbonate complexes (WHO, 2006). Similarly, *in vivo*, the bioavailability of free Co(II) is expected to be relatively limited because these cations precipitate in the presence of physiological concentrations of phosphates [$Co_3(PO_4)_2$; K_{sp}: 2.5×10^{-35} at 25 °C] and bind to serum proteins, such as albumin (IARC, 2006; Lison, 2007).

Dietary intake and nutritional importance

Only trace amounts of Co are required by the human body as a component of Vitamin B_{12}, and the recommended daily intake of Vitamin B_{12} in the US is 2.4 μg/d (IOM, 2012), corresponding to approximately 0.10 μg of Co. The average daily dietary intake of Co is highly variable, and depends largely on diet type and geographical location (Dabeka & McKenzie, 1995; Gal et al., 2008). The mean intake of Co has been estimated to be about 12 μg of Co/d, with normal dietary intake ranging between 5 and 40 μg of Co/d, with the highest

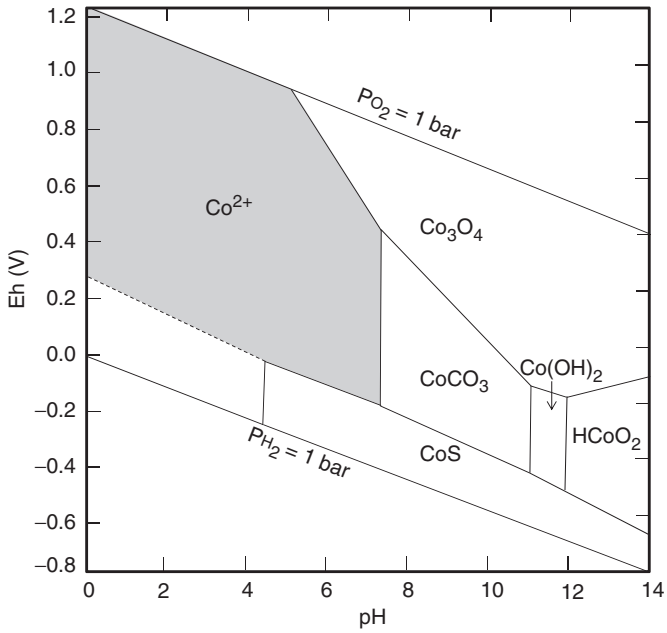

Figure 1. Eh-pH diagram for part of the system Co-S-C-O-H. Assumed activities for the dissolved species are the following: $Co = 10^{-6}$, $C = 10^{-3}$, $S = 10^{-3}$. Solubility increases as pH decreases. Adapted from Eh–pH diagrams for geochemsitry, Brookins (1988, p. 72).

concentrations of Co found in fish (0.01 mg/kg), fresh cereals (0.01 mg/kg), nuts (0.09 mg/kg) and green leafy vegetables (such as broccoli and spinach: 0.009 mg/kg) (Biego et al., 1998; Gal et al., 2008; Health Canada, 2007; Hokin et al., 2004; IARC, 2006).

Co supplements in the form of Co(II) are readily available in the US, and some manufacturers have recommended daily doses as high as 1 mg Co/d to help with fat and carbohydrate metabolism, protein synthesis and RBC production (DRN, 2012; MEMI, 2011; Mineralife, 2012).

Toxicology studies of cobalt

Acute human studies

There are a few case reports pertaining to the acute toxicity of Co following oral ingestion. An autopsy of a 19-month-old male child who died after ingesting approximately 30 mL of $CoCl_2$ revealed coagulative necrosis of the stomach mucosa, and microscopic examination of the brain revealed edema (Jacobziner & Raybin, 1961). It was noted that collectively the child's liver, kidneys and spleen contained 89.4 mg Co, approximately two orders of magnitude greater than the typical total body content of about 1.1 mg Co (Jacobziner & Raybin, 1961).

Co plasma and whole blood concentrations of 426 and 237 μg/L, respectively, were reported in a 6-year-old boy a few hours after he drank approximately 2 g of $CoCl_2$ that he added to a blackcurrant cordial. The boy complained of abdominal pain, vomited and was given an emetic 4 h after ingesting the Co. Physical examination and cardiac monitoring were normal, and even at this very high dose the Co was cleared rapidly from the plasma and whole blood (Table 2) (Mucklow et al., 1990).

Co whole blood concentrations were also monitored in an accidental poisoning case caused by Co released to juice contained within a Co-blue glazed jug. The first blood analysis indicated a Co concentration of 16 μg/L. After two weeks of chelator treatment, the patient's blood Co concentration dropped to ~1 μg/L, and, five weeks later, the Co was <1 μg/L. The patient showed no cardiac toxicity, and it was estimated that she was exposed to 9 to 36 mg of Co daily for approximately 3 months (Selden et al., 2007).

Table 2. Plasma and whole blood cobalt concentrations in a 6-year-old male following ingestion of approximately 2 g of $CoCl_2$. It is important to note how quickly Co levels in plasma and whole blood decrease after the poisoning (Mucklow et al., 1990).

	Plasma (μg/L)	Whole blood (μg/L)
Day of poisoning	426	237
2 d after poisoning	80	35
5 d after poisoning	50	15
10 d after poisoning	19	12
14 d after poisoning	13	9
17 d after poisoning	9	6
26 d after poisoning	5	5

Subchronic and chronic human studies

Human subchronic and chronic oral exposure to Co has been associated with effects on the hematological, thyroid and cardiovascular systems. Some case reports have also indicated the occurrence of reversible neurological responses. Much of this information is taken from studies conducted in the 1950s, when Co was marketed under the name Roncovite® for treating various forms of anemia in both children and adults. Not surprisingly, the therapeutic doses were generally lower than those used in the animal toxicology studies. In many of the reports, it is possible to estimate no-effect dose levels (and blood concentrations) for certain disease endpoints (Finley et al., 2012a). Where possible, we identify the no observed effect level and the lowest observed effect level doses in these studies.

Hematological effects

Davis & Fields (1958) exposed six healthy men aged 20–47 years to a 2% $CoCl_2$ solution for up to 22 days. Five of the six men received 150 mg $CoCl_2$/d (68 mg Co/d) for the entire exposure period, while the sixth started on 120 mg $CoCl_2$/d (54 mg Co/d) and later received 150 mg $CoCl_2$/d. At this dose (~1 mg Co/kg-d), all six subjects reportedly developed polycythemia (increased RBC counts above six million). On the other hand, Jaimet & Thode (1955) dosed 15 young children (aged 5–9 yr) with similar and higher doses (0.45, 0.90 or 1.8 mg Co/kg-d for 10 weeks), yet no clinically significant increase in blood hemoglobin levels was observed at any dose. Similarly, Holly (1955) reported no alterations in hemoglobin levels following Co administration (0.53 mg Co/kg-d) to 20 pregnant women. The no observed effect level doses and the lowest observed effect level doses from these studies are presented in Table 3; blood Co concentrations associated with hematological health endpoints are presented in Figure 2. Several occupational studies have evaluated blood Co concentrations and hematological effects in Co-exposed workers (Angerer et al., 1985; Lantin et al., 2011; Raffn et al., 1988; Swennen et al., 1993). As shown in

Table 3. Various hematological studies of human populations exposed to various concentrations of cobalt and the dose at which a hematological effect was or was not observed.

Reference	Exposed group	Response reported	Response category	No effect dose (mg Co/kg-d)	Effect dose (mg Co/kg-d)
Holly et al. (1955)	20 pregnant women	No hematological alterations	Hematological	0.53	
Jaimet & Thode (1955)	18 children (10 male and 8 female)	No change in hemoglobin levels	Hematological	1.8	
Davis & Fields (1958)	6 healthy adult males	Polycythemia	Hematological		0.97
Bowie & Hurley (1975)	11 adult dialysis patients	Increased hematocrit and RBC volume	Hematological		0.32
Duckham & Lee (1976)**	4 adult dialysis patients	Increased hemoglobin levels	Hematological		0.18
Angerer et al. (1985)	40 foundry workers	No effects on erythropoesis	Hematological	NA*	
Raffn et al. (1988)	46 plate painters	Decreased hematocrit and mean cell volume, no changes in hemoglobin and RBCs	Hematological	NA*	
Swennen et al. (1993)	82 foundry workers	Decreased RBCs, hemoglobin and hematocrit	Hematological	NA*	
Lantin et al. (2011)	249 foundry workers	No change in RBCs	Hematological	NA*	

*Study reported blood or serum concentration, but a dose could not be determined from the available information.
**A total of eight patients were treated but Co serum concentrations were only reported for four of the patients; as such only those four patients were included in the analysis conducted by Finley et al. (2012a).

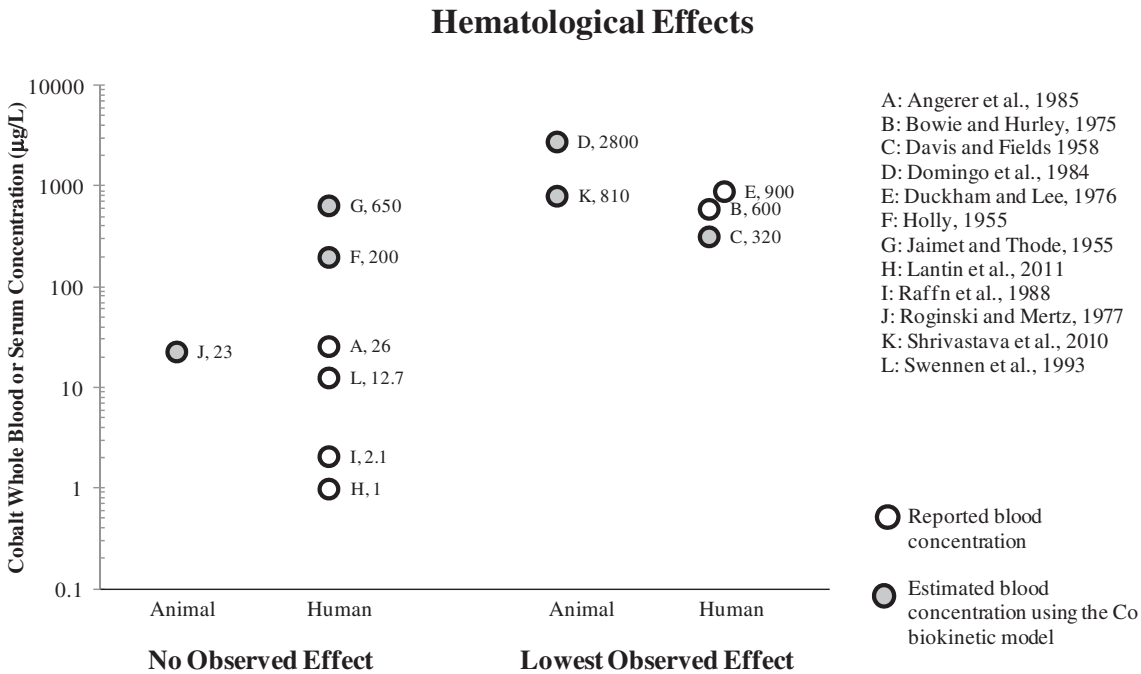

Figure 2. Blood Co concentrations associated with hematological health endpoints. Adapted from Finley et al. (2012a).

Figure 2, the average measured Co blood concentrations in these cohorts ranged from 1 to 26 µg/L, yet no hematological effects (change in RBC or hematocrit levels) were noted.

Thyroid effects

As summarized in Table 4 and Supplementary Table 2, several studies reported that oral Co doses ranging between 0.11 and 10 mg Co/kg-d decreased iodine uptake by the thyroid, which sometimes resulted in a goiter and classic signs of hypothyroidism. For instance, Chamberlain et al. (1961) described the development of goiters in two infants and one toddler undergoing Co therapy. Roche & Layrisse (1956) reported that oral Co ingestion at 1 mg Co/kg-d as $CoCl_2$ in healthy adults for two weeks inhibited radioactive iodine uptake in the thyroid (radioiodine thyroidal uptake tests were less than 20% of the administered dose). When Co treatment ended, iodine uptake returned to normal. Additionally, Paley et al. (1958) reported decreased iodine uptake by the thyroid in two of four patients exposed to 0.54 mg Co/kg-d (oral dose) for 10–21 d. Bowie & Hurley (1975) reported no thyroid effects (as measured by changes in serum thyroxine and TSH levels) in 11 dialysis patients taking 11.3 mg Co for four weeks, followed by 22.6 mg Co for four weeks with measured blood Co concentrations ranging from 220 to 2100 µg/L at the end of eight weeks of treatment (mean of ~600 µg/L, Figure 3). It is important to note that in almost all cases, the thyroid effects were reversible following exposure cessation.

Interestingly, some studies involving children appear to suggest a dichotomous response. Specifically, as shown in Table 4, clinical treatment of sickle cell anemia in children with doses ranging from 1.4 to 1.8 mg Co/kg-d for up to 7 months was associated with decreased iodine uptake and

Table 4. Various thyroid studies of human populations exposed to various concentrations of cobalt and the dose at which a thyroid effect was observed.

Reference	Exposed group	Response reported	Response category	No effect dose (mg Co/kg-d)	Effect dose (mg Co/kg-d)
Jaimet & Thode (1955)	18 children (10 male and 8 female)	Decreased iodine uptake	Thyroid	1.8	2.7
Roche & Layrisse (1956)	12 adults (gender not specified)	Decreased iodine uptake	Thyroid		0.97
Bowie & Hurley (1975)	11 adult dialysis patients	No changes in serum thyroxine and TSH	Thyroid	0.32	
Paley et al. (1958)	4 adults (3 males and 1 female)	Decreased iodine uptake	Thyroid		0.54
Swennen et al. (1993)	82 foundry workers	No changes in T3 uptake, T4, or TSH, decrease in total T3	Thyroid	NA*	
Lantin et al. (2011)	249 foundry workers	No changes in serum T3, T4 and TSH	Thyroid	NA*	
Gross et al. (1955)	4 children	Goiters and decreased iodine uptake	Thyroid		1.4
Kriss et al. (1955)	4 children and 1 adult	Goiters and decreased iodine uptake	Thyroid		1.4

*Study reported blood or serum concentration, but a dose could not be determined from the available information.

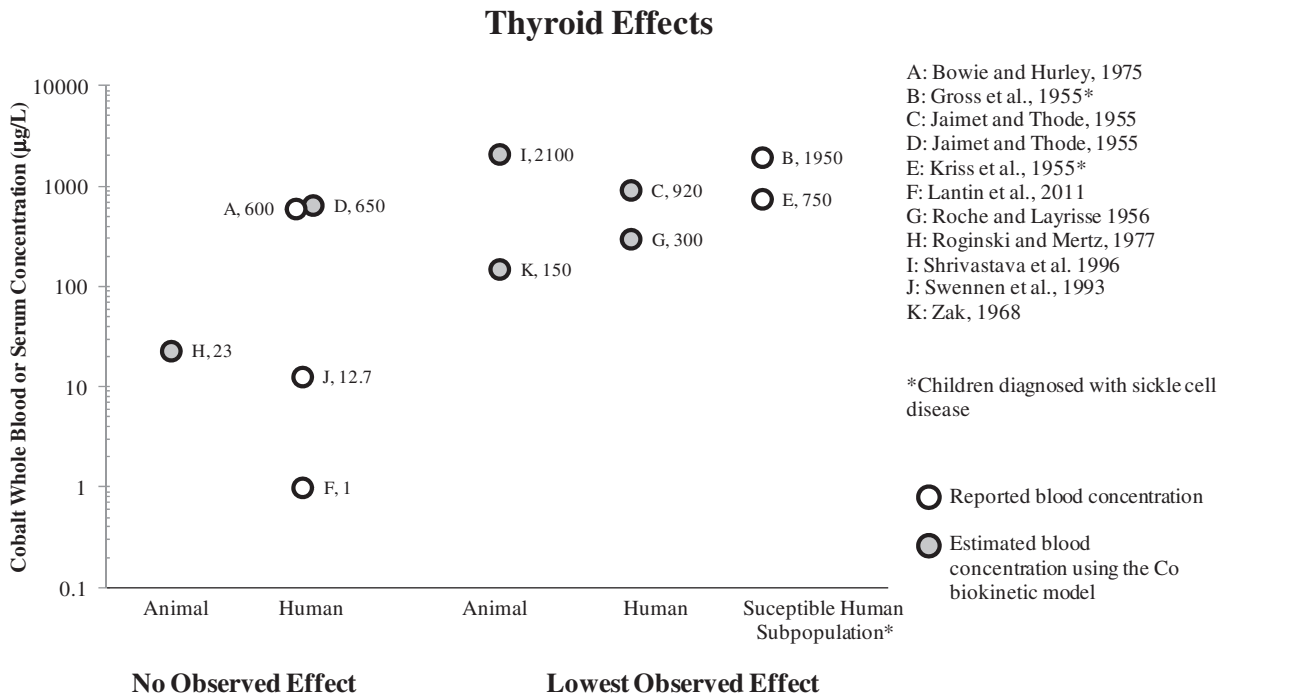

Figure 3. Blood Co concentrations associated with thyroid health endpoints. Adapted from Finley et al. (2012a) with the addition of Kriss et al. (1955) and Gross et al. (1955).

development of goiters (Gross et al., 1955; Kriss et al., 1955). Conversely, Jaimet & Thode (1955) dosed four groups of 4–5 young children without sickle cell anemia (18 children total, aged 5–9) with 0.45, 0.90, 1.8, or 2.7 mg Co/kg-d for 10 weeks and no thyroid effects were noted at doses of 0.45–1.8 mg/kg-d (decreased iodide uptake was noted in two of the five children after five weeks of dosing at 2.7 mg Co/kg-d). These findings suggest that sickle cell anemia patients may have higher concentrations of free Co which may increase their risk of adverse effects at sufficient doses (Figure 3).

Neurological effects

Certain neurological effects, such as reversible hearing and vision impairment, have been reported in a few anephric patients undergoing Co therapy for chronic anemia associated with kidney disease (Bowie & Hurley, 1975; Curtis et al., 1976; Duckham & Lee, 1976; Gardner, 1953; Kriss et al., 1955; Manifold et al., 1978; Schirrmacher, 1967; Schleisner, 1956). Most of these reports indicate that the effects were reversible after ceasing Co therapy (Table 5; Figure 4; Supplementary Table 1) (Bowie & Hurley, 1975; Gardner, 1953; Schirrmacher, 1967). In some instances, these anephric patients experienced more sensitive responses, such as polycythemia and thyroid dysfunction.

The reports of Bowie & Hurley (1975) and Duckham & Lee (1976) are unique because they are the only patient dosing studies that also reported measured serum Co concentrations (but in patients with kidney problems). The reported serum Co concentrations associated with neurological effects were very high. For instance, partial hearing impairment (changes greater than 10 decibels over 6000 Hz) was reported in three hemodialysis patients with Co serum concentrations of 560, 600 and 2100 µg/L (average was 1087 µg/L; Figure 4) at the end of eight weeks of treatment (four weeks at 25 mg $CoCl_2$ followed by four weeks at 50 mg $CoCl_2$) (Bowie & Hurley, 1975). Peak Co serum

Table 5. Various neurological studies of human populations exposed to various concentrations of cobalt and the dose at which a neurological effect was observed.

Reference	Exposed group	Response reported	Response category	No effect dose (mg Co/kg-d)	Effect dose (mg Co/kg-d)
Bowie & Hurley (1975)	12 adult dialysis patients	Reversible hearing loss	Neurological	0.32	0.32**
Duckham & Lee (1976)***	4 adult dialysis patients	No detectable nerve damage or polyneuropathy	Neurological	0.18	
Meecham & Humphrey (1991)	1 adult occupationally exposed to Co	Reversible vision and hearing loss	Neurological		NA*

*Study reported blood or serum concentration, but a dose could not be determined from the available information.
**Peak Co serum concentrations reported for these three patients during dosing were 820, 1620 and 2100 µg/L.
***A total of eight patients were treated but Co serum concentrations were only reported for four of the patients; as such only those four patients were included in the analysis conducted by Finley et al. (2012a).

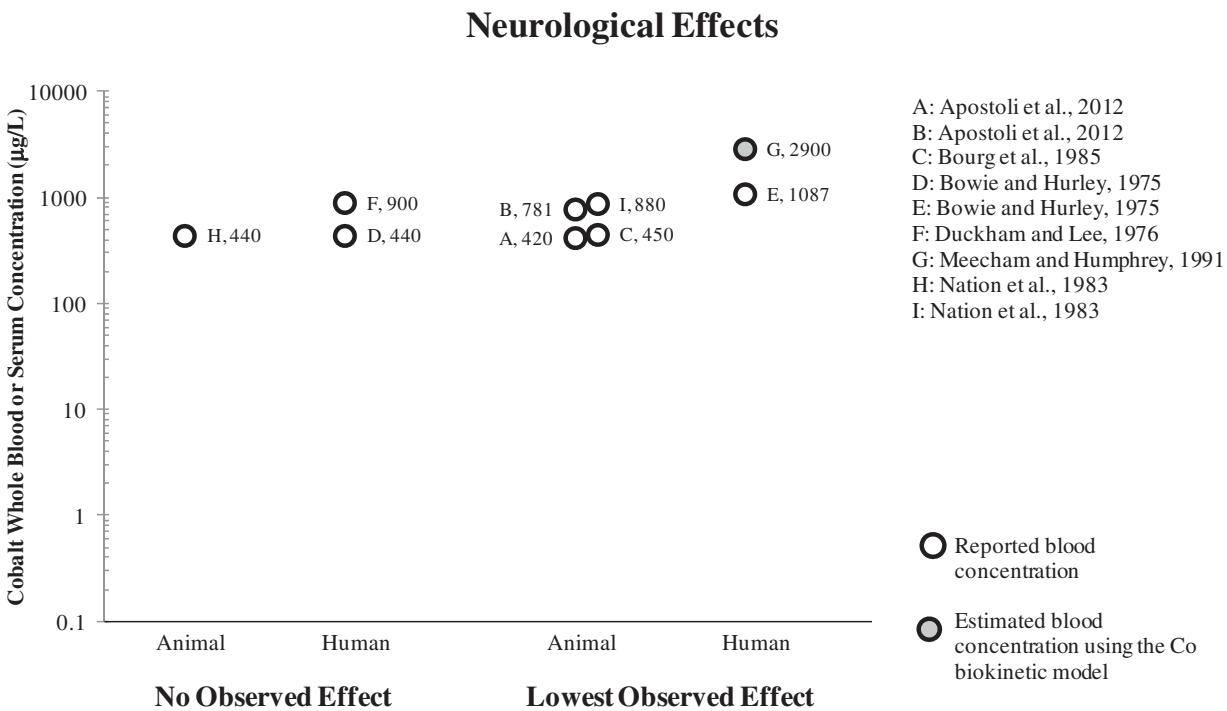

Figure 4. Blood Co concentrations associated with neurological health endpoints. Adapted from Finley et al. (2012a).

concentrations reported for these three patients during dosing were 820, 1620 and 2100 µg/L. In this same group, no such effects were noted in nine patients with Co serum concentrations ranging from 220 to 570 µg/L (mean of 440 µg/L; Figure 4) at the end of eight weeks of treatment; peak Co serum concentrations reported during dosing ranged from 220 to 610 µg/L (Bowie & Hurley, 1975).

Duckham & Lee (1976) evaluated auditory acuity and peripheral neuropathy in anephric patients undergoing Co therapy to treat anemia. During the 12 weeks of Co therapy (12.36 mg Co/d; an estimated dose of 0.18 mg Co/kg-d, assuming a 70 kg body weight), Co serum concentrations in four patients ranged from 640 to 1220 µg/L (mean of 900 µg/L; Figure 4), and the authors noted that ''. . . none of our patients suffered any clinically detectable eighth-nerve damage. Similarly no patient developed peripheral neuropathy'' (Duckham & Lee, 1976). It was noted that one patient developed ''slight'' high tone deafness after an additional 40 weeks of Co therapy (12.36 mg Co/d for 23 weeks followed by 6.18 mg Co for 17 weeks, assuming for a 70-kg adult this equates to ~0.18 and 0.09 mg Co/kg-d, respectively); his Co serum concentrations were reported to range from 420 to 490 µg/L during the end of this time period and peaked at 940 µg/L during the third month of the treatment (Duckham & Lee, 1976).

The blood Co concentrations reported by Duckham & Lee (1976) and Bowie & Hurley (1975) were much higher than would be expected in patients with functioning kidneys, because these patients were unable to efficiently remove Co via the renal system. For example, Curtis et al. (1976) reported that Co blood concentrations in hemodialysis patients treated with 50 mg $CoCl_2$ daily for two weeks peaked around 400 to 800 µg/L at the end of the second week of therapy, whereas the Co blood concentration in healthy patients following the same treatment peaked around 100 µg/L at the end of the second week. Thus, those on hemodialysis tend to have 4-8 fold higher Co blood concentrations, at a given dose, versus normal adults due to their inability to rapidly clear Co.

Cardiomyopathy

Several studies of cardiovascular effects in Co-exposed occupational cohorts have been published, and many of these report blood Co concentrations as well. Specifically,

Table 6. Various cardiac studies of human populations exposed to various concentrations of cobalt and the dose at which a cardiac effect was observed.

Reference	Exposed group	Response reported	Response category	No effect dose (mg Co/kg-d)	Effect dose (mg Co/kg-d)
Jacquet (1949)	Hypertension patients	No cardiac effects	Cardiac	0.11	
Kesteloot et al. (1968)	12 beer drinkers	No cardiomyopathy	Cardiac	0.09	0.09**
Morin et al. (1971)	50 beer drinkers	Cardiomyopathy	Cardiac		0.04
Alexander (1972)	28 beer drinkers	Cardiomyopathy	Cardiac		0.07
Bonenfant (1969)	20 beer drinkers	Cardiomyopathy	Cardiac		0.07
Angerer et al. (1985)	40 foundry workers	No cardiomyopathy	Cardiac	NA*	
Raffn et al. (1988)	46 plate painters	No differences in electrocardiography	Cardiac	NA*	
Swennen et al. (1993)	82 foundry workers	No serum changes in myocardial protein kinase	Cardiac	NA*	

*Study reported blood or serum concentration, but a dose could not be determined from the available information.
**Effect only observed at this dose in malnourished alcoholics.

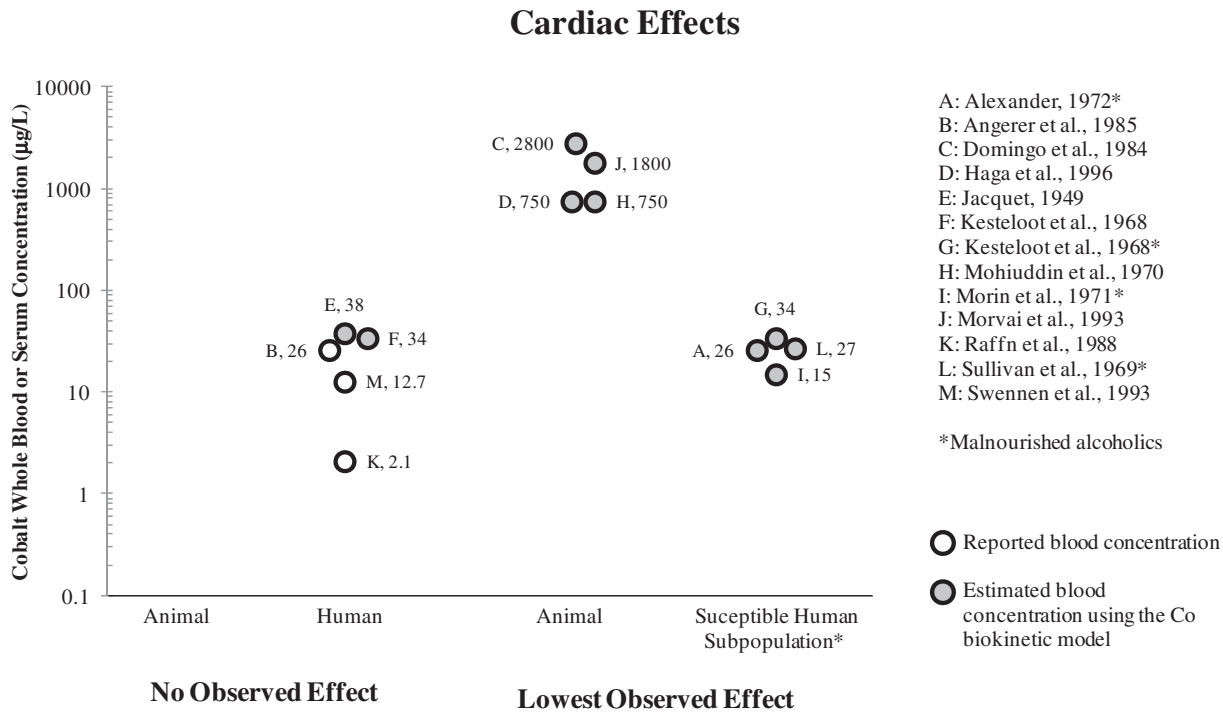

Figure 5. Blood Co concentrations associated with cardiac health endpoints. Adapted from Finley et al. (2012a).

Angerer et al. (1985), Raffn et al. (1988) and Swennen et al. (1993) reported no cardiovascular effects (e.g., no cardiomyopathy or changes in electrocardiography) in workers with mean blood Co concentrations ranging from 2.1 to 26 µg/L (Figure 5). In addition, Jacquet (1949) reported no cardiac effects in patients treated for hypertension with 6.8–9.1 mg Co/d (0.097–0.13 mg Co/kg-d).

In the 1960s, a unique Co-related cardiomyopathy syndrome was reported in a subset of heavy beer drinkers who consumed large quantities of beer in which $CoSO_4$ or $CoCl_2$ had been added as foam stabilizers. The beer drinkers ingested an average of 0.04 to 0.14 mg Co/kg-d for months to perhaps 2 years (Table 6; Figure 5). The cardiomyopathy was characterized by an enlarged heart, left ventricular failure, diminished myocardial compliance and pericardial effusion, as well as by extensive intracellular changes, including alterations in the myofibrils, glycogen and cellular mitochondria. The disease was fatal in approximately 43% of a case series of affected beer drinkers (Alexander, 1969, 1972; Morin et al., 1971). However, the population of all exposed ''Co beer drinkers'' was not fully characterized, so the incidence rate for cardiomyopathy over the entire exposed population remains unclear.

Importantly, Kesteloot et al. (1968) found that well-nourished beer drinkers (caloric intake between 2.180 to 3.290 kcal) experienced no cardiomyopathic effects at very similar doses (an estimated dose of 0.09 mg Co/kg-d, assuming a 70 kg body weight) compared to malnourished beer drinkers. Hence, as with the differences in susceptibility to thyroid responses observed in healthy versus sickle cell children, there is clearly a bimodal response in the heavy beer drinkers that appears to be related to nutritional status and perhaps to the severity of underlying liver and heart disease conditions related to chronic, severe alcoholism. A few other case reports of Co-induced cardiomyopathy have been reported in patients with end-stage renal disease (Curtis et al., 1976; Kriss et al., 1955; Manifold et al., 1978). Reports of Co-related cardiovascular effects in healthy individuals are rare, and it is possible that such effects simply do not occur unless a person's health is severely compromised in

conjunction with severely perturbed Co kinetics (i.e., reduced Co-albumin-binding capacity and/or enhanced equilibrium towards sustained, high Co(II) concentrations in blood and tissues).

Reproductive effects

To date, no studies regarding reproductive effects in humans following Co exposure have been reported. However, pregnant women have been treated with doses that ranged from 75 to 100 mg $CoCl_2$ daily (~34–45 mg Co/d) for up to 6 months corresponding to model predicted Co whole blood concentrations of 170–890 μg/L assuming a 15% to 35% GI absorption (Holly, 1955). No developmental effects on human fetuses were reported following the treatment of pregnant women with $CoCl_2$ to elevate hemoglobin and hematocrit levels during pregnancy (Holly, 1955, 1957), and no disturbance of thyroid or liver function was reported in any of the mothers or offspring of pregnant mothers who received 45 to 60 mg $CoCl_2$ daily (~20–27 mg Co/d) for a mean therapy time of 15 weeks (Holly, 1957). Unfortunately, no long term follow-up studies were conducted on these cohorts. Recently, Fritzsche et al. (2012) described a female patient with bilateral metal-on-metal (MoM) hip arthroplasties who had elevated Co blood concentrations throughout pregnancy (138–143 μg/L) and gave birth to a healthy male infant at 38 weeks of gestation. At the age of 8 weeks, the infant's Co blood concentration was 13 μg/L. It was noted that the infant's development after 14 weeks was ''uneventful'' (Fritzsche et al. 2012). Similarly, no teratogenic effects were observed in three pregnant patients with MoM hip resurfacings (deSouza et al. 2012).

Dermatological effects

Based on a review of the published literature describing the therapeutic use of Co in the past, skin rashes, pimples, dermatitis and dermal flares were described as adverse reactions following oral administration of Co at various doses (Davis & Fields, 1958; Holly, 1955; Sidell et al., 1958). For example, Sidell et al. (1958) reported 60 cases of ''pronounced activation of acne'' in patients receiving $CoCl_2$. The dose and total duration of exposure were not reported; but it was noted that this reaction occurred primarily in females after 1–10 weeks of exposure, and that the acne developed over the face and trunk. The authors reported that the dermal manifestations cleared up spontaneously over a course of 4–6 weeks (some cases were more persistent) after the use of Co was discontinued (Sidell et al., 1958). Kasanen et al. (1962) treated 36 women and four men with 3 mg of Co intramuscularly every day or every other day for a total of six treatments; one of the patients reportedly developed a severe skin rash that continued for 3 months, but the authors noted that there was ''no plausible reason'' to account for the rash (Kasanen et al., 1962).

Stuckert & Nedorost (2008) estimated that 1% of patients with dyshidrotic eczema may develop a flare of dermatitis with Co exposure equal to the average dietary intake (Stuckert & Nedorost, 2008). Veien et al. (1987) evaluated flares of dermatitis in several patients with eczema of the hands, who were challenged orally with a single dose of 1 mg Co as $CoSO_4$. The authors reported a statistically significant difference in reactivity, qualitatively measured as a ''flare of dermatitis,'' between the placebo group and the group that received Co. However, whether a ''flare of dermatitis'' is a sufficiently specific or a reliable immunologic response is difficult to determine. It is important to note that the study population was also challenged with 2.5 mg Ni as $NiSO_4$, a known strong sensitizing agent, and then 1 week before or after dosing was challenged with 1 mg Co. Thus, some people who showed a response to Co may have been cross-sensitized by Ni exposure.

In short, infrequent skin reactions, including temporary acne and rashes, have been associated with Co therapy; in most cases these skin reactions were reportedly mild in severity, and other risk factors (e.g., being female and previous acne or dermatitis) may interact to modulate these effects.

Summary

It has been recognized since the 1950s that a small proportion of patients treated with Co developed nonserious adverse effects at various doses. Thyroid and hematological effects are the most sensitive responses. In healthy individuals, it appears that neurological and cardiomyopathic effects only occur at much higher doses. For some adverse effects (e.g., cardiomyopathy in adult beer drinkers and thyroid effects in sickle cell children), there was a differential response possibly related to underlying disease states and/or protein malnutrition.

Correlation of human and animal toxicity studies

Although the focus of this review is on the systemic toxicology of Co in humans, correlations between the human and animal data as to the range of adverse effects and with respect to dose–response relationships are important to consider. For interested readers, a more detailed description of the studies of animals exposed to Co is provided in the Supplemental Materials, Appendix A.

Rat inhalation studies of Co metal powder exposures at low concentrations (2.1–2.7 mg/m^3) for 5 h to 4 d identified little or no evidence of lung toxicity (Kyono et al., 1992). Inhalation exposures to rats at 7 mg Co/m^3 as Co hydrocarbonyl for 30 min were reported to have no effect; a slight increase in lung damage was reported at 26 mg/m^3, and severe edema was noted at 83 mg/m^3 (Palmes et al., 1959). In a 16 d inhalation study, exposure to 42 mg Co/m^3 as cobalt sulfate heptahydrate ($CoSO_4 \cdot 7(H_2O)$) resulted in the death of all rats and mice within the first 5 d; partial survival was observed following exposure to 10.5 mg Co/m^3 (NTP, 1991). In addition, severe inflammatory changes in both rats and mice following exposure to 10.5 mg Co/m^3 were noted and necrosis in the thymus and liver were reported in rats and mice that died during the exposure period.

Several reports on the acute oral toxicity of different forms of Co have been examined at relatively high doses. Increased diarrhea and ataxia were reported in rats following exposure to a single $CoSO_4$ dose of 209 mg Co/kg (ATSDR, 2004); mild ($CoSO_4$) or moderate ($CoCl_2$) motor activity reduction was reported in rats following a single exposure to 35 mg

Co/kg or 7.8 mg Co/kg, respectively (Singh & Junnarkar, 1991); hypothermia (110 mg Co/kg) and degenerative changes to the liver and heart (176 mg Co/kg) were reported after a single CoF_2 dose in rats, and hypothermia and renal/hepatic hyperemia (157 mg Co/kg) were reported in rats after a single CoO dose (Speijers et al., 1982). Overall, these studies highlight differences in the bioavailability of Co from the different Co compounds as illustrated by the varying degrees of acute toxicity. It is likely that the more toxic compounds elicit responses at lower doses due to greater bioavailability.

Subchronic and chronic animal studies have identified hematological, thyroid, optic/auditory neuropathy and myocardial effects following Co exposure. For example, increased RBCs, hemoglobin and hematocrit were reported in rats receiving daily $CoCl_2$ doses as low as 12.5 mg Co/kg-d for 7 d (Domingo et al., 1984; Shrivastava et al., 2010), and histopathological changes in the thyroid gland of mice were reported after 15–45 d of oral exposure to 48 mg Co/kg-d (Shrivastava et al., 1996). A 13-week inhalation study (NTP, 1991) reported perturbations in thyroid hormones at 10 and 30 mg Co/m^3 (13 wk × 5 d/wk × 6 h/d) as $CoSO_4$ in rats; thyroid function tests were not performed in mice.

Apostoli et al. (2012) measured whole blood Co concentrations in rabbits treated with approximately 0.16 mg Co/kg-d for 18 d or an average of 0.20 mg Co/kg-d for 53 d as $CoCl_2$ via intravenous infusion. The average reported whole blood Co concentration in rabbits following 18 d of exposure was 420.9 μg/L (ranged 304.5–639.8 μg/L), and an average whole blood Co concentration of 781.14 μg/L (ranged 586.2–1025.3 μg/L) was reported following 53 d of exposure. It was noted that both groups of rabbits suffered from optic toxicity, while rabbits treated with the higher Co dose for a longer exposure duration also suffered from auditory system toxicity (Apostoli et al., 2012).

Mohiuddin et al. (1970) reported cardiac effects in guinea pigs dosed with 7.6 mg Co/kg-d as $CoSO_4$ in their diet or via oral gavage with or without ethanol for 5 weeks. Grice et al. (1969) reported myocardial degeneration and high mortality in rats that were fed protein-free diets for 8 weeks and then given an initial oral dose of 100 mg Co/kg as $CoSO_4$, followed by daily oral doses of 26 mg Co/kg for 8 weeks (Grice et al., 1969). Other studies in dogs with thiamine-deficient diets (Sandusky et al., 1981) and in rats with ethanol co-administration (Morvai et al., 1993) also provided evidence suggestive of cardiomyopathy at similar or higher daily oral doses. Notably, these chronic animal studies identify the key adverse Co effects on hematopoiesis, thyroid function, optic/auditory nerves, and the myocardium that have been identified in humans following excessive systemic doses.

NTP (1998) reported a 2-year cancer bioassay on $CoSO_4 \cdot 7H_2O$ that found it to be carcinogenic in $B6C3F_1$ mice and F344/N rats following an inhalation exposure. Animals were exposed to 0.3, 1 or 3 mg/m^3 $CoSO_4 \cdot 7H_2O$ for 6 h/d, 5 d/week for 105 weeks. The authors reported there was a clear evidence of carcinogenicity in male mice (3 mg/m^3), female mice (1 or 3 mg/m^3) and female rats (1 or 3 mg/m^3) based on increased incidences of alveolar/bronchiolar neoplasms; the spectrum of lesions observed within the lungs of exposed rats and mice was broad. In addition, female rats had an increased incidence of pheochromocytoma (tumor) of the adrenal medulla at 3 mg/m^3. The authors reported that there was some evidence of carcinogenicity in male rats based on an increased incidence of lung tumors at the highest exposure levels (NTP, 1998). In addition, the incidence of follicular cell hyperplasia of the thyroid gland was reported to be moderately increased in all exposed groups of male mice, but no dose–response relationship was noted. In male and female rats, the incidence of neoplasms in the liver, cardiovascular system, nervous system, thyroid gland and thymus of exposed animals was similar to chamber controls. In male and female mice, the incidence of neoplasms in the cardiovascular system, thyroid gland and thymus of exposed animals was also similar to chamber controls.

Some animal studies have associated Co exposure with adverse reproductive or developmental effects following relatively high oral doses. Pedigo et al. (1988), for example, reported a dose-dependent decrease in testicular weight and epididymal sperm concentration in male mice exposed to 23, 42 or 72 mg Co/kg-d as $CoCl_2$ in drinking water over a 12-week period (Pedigo et al., 1988). A time-dependent decrease was also noted in epididymal sperm concentration, sperm motility and testicular weight in animals exposed to 72 mg Co/kg-d (Pedigo et al., 1988). Consistent with these findings, Anderson et al. (1993) reported that continuous oral exposure to $CoCl_2$ at 43 mg Co/kg-d in drinking water for 13 weeks resulted in seminiferous tubule degeneration in mice (Anderson et al., 1993). Nation et al. (1983) reported testicular atrophy in rats exposed to 20 mg Co/kg-d in their feed; no atrophy was observed at 5 mg Co/kg-d. NTP (1991) reported decreases in body weight, sperm motility and testicular atrophy in male mice at inhaled $CoSO_4$ doses of 3 mg Co/m^3 and higher (13 wk × 5 d/wk × 6 h/d). Elbetieha et al. (2008) reported decreases in sperm count, testicular weight, fertility implantation sites and fetal viability in mice exposed to 11.6 mg Co/kg-d, 21.3 mg Co/kg-d or 42.2 mg Co/kg-d as $CoCl_2$ via drinking water for 12 weeks.

In addition, studies in rats have reported that Co can cross the placenta (Palmen, 2005; Szakmary et al., 2001). Gestational exposures to Co were reported to result in stunted growth (5.4, 10.8 or 21.8 mg Co/kg-d) and reduced survival of offspring (21.8 mg Co/kg-d) (Domingo et al., 1985) following fairly high dosing. While the authors did not specifically describe toxic effects in the mothers, it was noted in previous studies that exposure to 10.8 and 21.8 mg Co/kg-d was associated with toxicity of the exposed animal, and thus the authors concluded that Co administration at these doses produced toxic effects on the mothers that had repercussions on the offspring (Domingo et al., 1985). In similar rat gestational exposure studies, significant growth retardation in the fetuses was reported following exposure to 5.2–21 mg Co/kg-d but there was no significant effect on maternal body weight relative to controls (Szakmary et al., 2001); where as, a non statistically significant increase in stunted fetuses was observed after exposure to 12 and 25 mg Co/kg-d and there was a significant decrease in maternal body weight observed in this study (Paternain et al., 1988). These doses expressed as their human equivalent dose (1.3-6.0 mg Co/kg-d) are approximately equal to Co whole blood concentrations of

480–5400 μg/L assuming a 15% or 35% oral absorption for nine months of exposure.

Little information is available on the potential adverse effects of Co on the immune system in animals. One study reported thymic atrophy in rats exposed to 4.2 mg Co/kg-d for 4 weeks (Chetty et al., 1979). Another study noted a deterioration in immunological reactivity, manifested by a decline in phagocytic activity, in rats following 6–7 months of treatment with 0.5 mg Co/kg-d (six days a week) or greater (Krasovskii & Fridliand, 1971). However, the authors did not present data on this endpoint.

A few animal studies have indicated that exposure to Co can result in kidney and liver damage at sufficient doses. Renal injury, as indicated by histological alterations of the proximal tubules, was reported in rats after exposure to 4.5 mg Co/kg-d via i.p. administration or 10 mg Co/kg-d via oral administration as $CoCl_2$ for 5.5 to 8 months, respectively (Holly, 1955; Murdock, 1959). The oral dose associated with reversible tubular necrosis, expressed as its human equivalent dose adjusted for 5 days a week of exposure (1.7 mg Co/kg-d), is approximately equal to Co whole blood concentrations of 650–1500 μg/L assuming a 15% to 35% oral absorption for 8 months of exposure. However, Franchini et al. (1994) reported that the kidney is not a target organ in humans during occupational exposure to Co, and this view is shared by others (ATSDR, 2004). In addition, Marker et al. (2008) found no clinically significant change in renal function, as indicated by serum creatinine levels and creatinine clearance rate, 10 years after total hip arthroplasty with a MoM Co-containing bearing.

Increased liver weight (17%) was reported in rats exposed to 4 mg Co/kg as $CoCl_2$, 5 d a week, for 7 months, but only a 9% increase in liver weight was reported in rats exposed to 10 mg Co/kg 5 d a week for 8 months (Murdock, 1959). No morphological or enzymatic changes were found in the livers of rats exposed to 2.5–30.2 mg Co/kg-d as $CoCl_2$ by gavage or via drinking water for 3–7 months (Domingo et al., 1984; Holly, 1955; Krasovskii & Fridliand, 1971).

Summary

In summary, the animal toxicology data indicate that, at sufficient doses, Co may elicit polycythemia, thyroid changes, optic/auditory neuropathy and myocardial damage consistent with observations in some studies of systemic Co toxicity in humans. Inhalation studies in animals have identified portal-of-entry inflammation and lung cancer at high doses, consistent with some human study observations. As expected, animal studies have also identified relatively high dose effects on other organs/systems (e.g., liver, kidney, immune system, and reproductive/developmental) that, to date, have not been reported in human studies. Thyroid and hematological effects appear to be the most sensitive systemic toxicity endpoints for Co in both animals and humans (Finley et al., 2012a).

Kinetics

Absorption

The gastrointestinal absorption of Co in humans has been reported to be about 25%, with large inter-individual variation ranging between 5% and 97% of the administered dose (Christensen et al., 1993; Elinder & Friberg, 1986; Harp & Scoular, 1952; IARC, 2006; Moshtaghie et al., 2004; Smith et al., 1972; Sorbie et al., 1971; WHO, 2006). The degree of gastrointestinal absorption of Co depends on multiple factors, including ingested dose, solubility of the compound and nutritional status of the individual. In general, very small doses of a few μg/kg are absorbed almost completely, whereas larger doses are less well-absorbed (Barceloux, 1999; Reuber et al., 1994; Stokinger, 1962, 1981; Taylor, 1962). These differences in uptake rate are also likely to affect Co distribution and excretion, but published pharmacokinetic data do not cover a broad range of dosing conditions.

Studies on the bioavailability of Co compounds indicate that highly water-soluble compounds, such as $CoCl_2$ are more readily absorbed relative to other inorganic Co compounds, likely related to enhanced uptake as Co(II) ions. For example, Christensen et al. (1993) measured the absorption of soluble $CoCl_2$ and insoluble Co_3O_4 in 12 male and 11 female volunteers who were administered 0.5 mg Co daily for 10 d. Based on median urinary excretion of Co, gastrointestinal uptake of the soluble $CoCl_2$ was ~14-times and 51-times greater in men and women, respectively, than the uptake of the insoluble Co_3O_4. The findings are consistent with several rat studies, indicating that soluble $CoCl_2$ is more readily absorbed (20%–34% of the orally administered dose) than insoluble Co-oxides (1%–3% of the orally administered dose) (ATSDR, 2004; Ayala-Fierro et al., 1999; Gregus & Klaassen, 1986; Reuber et al., 1994). Further, blood Co concentrations following oral administration of $CoCl_2$ ranged from 0.5 to 3 μg/L for men and 1.1 to 11 μg/L for women, suggesting that Co absorption was higher in females than in males. In support of this, urinary Co concentrations in women were significantly higher than in men (Christensen et al., 1993).

Co absorption is also influenced by nutritional factors that are likely associated with reduced uptake of Co(II) ions due to the formation of complexes with certain organic anions. For example, amino acids have been shown to reduce Co absorption, since both amino acids and sulfhydryl groups bind with Co ions (Elinder & Friberg, 1986). Taylor (1962) investigated the gastrointestinal absorption of Co in rats and reported that Co absorption was decreased by more than 50% when $^{58}CoCl_2$ was administered in conjunction with histidine and lysine, versus when $^{58}CoCl_2$ was administered alone. Further, Paley & Sussman (1963) reported that Co absorption in human volunteers was enhanced after fasting. Fe deficiency has also been reported to increase Co absorption in both animals and humans (Barceloux, 1999; Reuber et al., 1994; Sorbie et al., 1971; Valberg et al., 1969). For example, Barany et al. (2005) reported an inverse relationship between Fe status and Co blood concentrations in adolescents. Consistent with these earlier findings, a recent study by Meltzer et al. (2010) reported a significant correlation between low Fe status and increased blood Co concentrations in women. In short, the fraction of ingested Co absorbed through the gut appears to be influenced by time since last meal, nutritional status and Fe status.

These findings suggest that Co and Fe may share a common intestinal uptake mechanism that may be up-regulated with anemia or Fe deficiency. These two ions also

have very similar atomic diameters and valence characteristics that allow them to compete for the same biomolecular-binding sites in the body. For example, Co(II) ions and Fe(II) ions may compete for uptake by the divalent metal transporter, DMT-1, which is substantially up-regulated by dietary Fe restriction, or by increased Fe demand (as in an Fe deficient state) (Garrick et al., 2006; Kwong & Niyogi, 2009; Meltzer et al., 2010; Thomson & Valberg, 1972). In addition, natural resistance-associated protein 1 (Nramp1), which is expressed at the phagosomal membrane of macrophages and neutrophils, have been reported to mediate both Fe(II) and Co(II) uptake (Forbes & Gros, 2003). Thus, both DMT-1 and Nramp1 allows non-protein-bound divalent metals to enter cells (Forbes & Gros, 2003; Howitt et al., 2009).

The major protein carriers for Co(II) ions in blood are serum albumin and α_2-macroglobulin (Tietz & Andresen, 1986). Neilsen et al. (1998) also showed that Co(II) ions can bind to lipoproteins and haptoglobin, which binds the globin portion of free hemoglobin. In addition, Co(III) has been reported to bind to transferrin and decrease Fe binding (Moshtaghie et al., 2004). The relative importance of these active and passive transport/binding mechanisms for Co ions in blood and tissues remains poorly understood, and likely involves competitive interactions with receptor binding that affect body feedback systems involving other critically important divalent cations like Fe and Ca (Karovic et al., 2007).

Distribution

In humans, Co is distributed mainly to the serum, whole blood, liver, kidney, heart and spleen with lower concentrations found in the bone, hair, lymph, brain and pancreas (Collecchi et al., 1986; Elinder & Friberg, 1986; Forbes et al., 1954; Hewitt, 1988; Ishihara et al., 1987; Muramatsu & Parr, 1988; Teraoka, 1981; WHO, 2006; Yamagata et al., 1962; Yukawa et al., 1980). These tissue concentrations reflect exposure from all routes, and the total Co body burden in humans has been estimated to be about 1.1 mg, with approximately 85% of Co in the adult human body being present in the Vitamin B_{12} organometallic complex (Lison, 2007). Vitamin B_{12} is absorbed through a complex pathway requiring intrinsic factor secretion in the gastrointestinal tract, which is different from that of inorganic Co. Once absorbed into the body, Vitamin B_{12} is metabolized to methyl-cobalamin or deoxyadenosyl-cobalamin in cells, stored in the liver, and ultimately excreted in urine and feces (EGVM, 2003; Watanabe et al., 2007). Stored Co in the body does not appear to significantly accumulate with age (Goyer & Clarkson, 2001; Gregus & Klaassen, 1986; ICRP, 1979; Lison, 2007; Schroeder et al., 1967; Tipton & Cook, 1963; Yamagata et al., 1962). For example, Schroeder et al. (1967) examined the raw data of Tipton & Cook (1963) and Tipton et al. (1965) and concluded that there was no evidence that Co accumulated or depleted with age (Schroeder et al., 1967; Tipton & Cook, 1963).

Whole body radioisotope scans taken at various time points following intravenous Co exposure found that 10%–30% (mean 20%) of the total whole body content was found in the liver (Smith et al., 1972). Slightly higher liver Co concentrations were reported by Jansen et al. (1996), who administered radioactive Co (as $^{55}CoCl_2$) to two healthy male volunteers by intravenous injection and reported that Co distributed primarily to the liver and bladder, with 50% of the administered dose accumulating in the liver (Jansen et al., 1996).

Similarly, animal studies indicate that Co absorbed through the gastrointestinal tract is primarily retained in the liver, with smaller quantities found in the skeleton, kidney, heart, stomach and intestines (Ayala-Fierro et al., 1999; Barnaby et al., 1968; Greenberg et al., 1943; Persson et al., 1992; Simesen, 1939; WHO, 2006). For example, in rats, tissue data obtained during daily administration of Co through drinking water indicated that Co distributed primarily to the liver and kidney and, to a lesser extent, the heart (Thomas et al., 1976). These findings are consistent with other studies, including a 1968 study by Barnaby et al. that described the retention and distribution of radioactive $^{60}CoCl_2$ in rats 132 d after intravenous, intraperitoneal or oral administration. The liver was reported to initially contain the highest level of Co following oral or intraperitoneal administration (~4% and ~5% of the radioactivity administered, respectively), but after 132 d, the highest levels of Co were reported in the muscle (~0.03% and ~0.1%, respectively) and skeleton (~0.05% and 0.16% of dose, respectively) relative to other tissues (Barnaby et al., 1968).

The tissue partitioning and accumulation of Co is dependent upon the concentration of free Co ions that are biologically available versus bound Co that are less available. For Co concentrations in serum between background concentrations of 0.1 to 0.4 μg/L to concentrations up to 3000 μg/L, approximately 8.3% to 8.5% of Co is predicted to occur as free ionic Co in serum for a nominal albumin concentration of 44 g/L, while the rest is bound to serum proteins (primarily albumin) (Nandedkar et al., 1972). The fraction of free ionic Co is relatively constant at serum concentrations up to 3000 μg/L because these concentrations are well below the maximal-binding capacity of approximately 80 to 120 mg Co/L for a nominal albumin concentration of 44 g/L, and an assumption of 2 to 3 Co binding sites.

Excretion

Following oral administration, unabsorbed Co is primarily excreted via feces, while the absorbed fraction is primarily excreted via urine, and to a lesser extent, the feces (ATSDR, 2004; EFSA, 2009; Reuber et al., 1994). For instance, Smith et al. (1972) reported that, on average, 22% of the administered ^{60}Co dose was excreted in the urine and 1.8% of the administered dose was excreted in the feces 24 h after the intravenous administration of $^{60}CoCl_2$. Sorbie et al. (1971) also reported that within 24 h of oral administration of radioactive $CoCl_2$, 18% of the administered dose was excreted via the urine. Average Co retention for two subjects followed for approximately 1000 d after intravenous administration of ^{60}Co indicated that 44% of the administered dose leaves the body with a biological half-life of 0.5 d, 32% clears with a biological half-life of 6 d, 13% clears with a biological half-life of 60 d, and the last 11% is cleared with a biological half-life of approximately 800 d (Smith et al., 1972).

Letourneau et al. (1972) monitored whole body Co concentrations in 16 male subjects for approximately 1 year after intravenous injection of $^{58}CoCl_2$. On average, 36% of the administered dose was cleared with a biological half-life of 6 h, 24% with a half-life of just less than 2 d, 19% with a half-life of 8 d, 13% with a half-life of approximately 50 d and 9% with a half-life of about 600 d. Although Smith et al. (1972) estimated a slightly longer retention time than Letourneau et al. (1972), both studies show that a majority of the administered dose is cleared within 7 d, with 36%–44% of the dose being excreted within 6–12 h.

Consistent with human studies, animal studies have also shown that urinary excretion is the primary elimination route for absorbed Co and that Co excretion occurs very rapidly once exposure ceases (Andre et al., 1989; Bailey et al., 1989; Collier et al., 1989; Patrick et al., 1989; Talbot & Morgan, 1989). For example, in male rats, 62.9% of the administered dose was found to be excreted via the urine within 12 h following a single intravenous injection of 4.16 mg Co/kg (Ayala-Fierro et al., 1999). Gregus & Klaassen (1986) reported that in rats, 73% of the administered Co dose was excreted via the urine within 4 d following intravenous injection. Overall, both studies show that the total Co excretion was rapid with a majority of the administered dose being excreted via the urine within a few hours to a few days.

Pharmacokinetic models

Available pharmacokinetic models for Co include only relatively simple compartment models because the mechanisms controlling the behavior of Co in the human body are not well understood quantitatively, which, at this time, prevents the construction of a true physiologically based pharmacokinetic model (PB-PK). However, The International Commission on Radiological Protection (ICRP) developed a 3-compartment biokinetic model for systemic Co in humans following oral exposure that divides the absorbed Co as follows: 50% is excreted in urine and feces, 5% is transferred to the liver and 45% is transferred to other tissues (ICRP, 1979; 1993). Elimination from tissue compartments is described by three first-order rate constants representing slow, medium and fast elimination pools, with half-lives of 6, 60 and 800 d, respectively (ATSDR, 2004). The model does not provide a physiological (realistic) description of how Co moves through the body over time because it does not take into account different tissue transfer rates or the complex fate of bound versus free Co ion species, or specific influences of active transport mechanisms.

Leggett (2008) developed a biokinetic model for inorganic Co that consists of a blood compartment and separate compartments for the skeleton, liver, kidney and other soft tissues. The model uses tissue transfer coefficients to account for excretion and secretion of Co from various body compartments, and assumes first-order kinetics. The proposed systemic model for Co distribution in the body is depicted in Figure 6. While the Leggett model yields similar predictions to the total Co-body retention as the ICRP (1993) model, it yields significantly different predictions of the systemic distribution. Building on the Leggett (2008) model, Unice et al. (2012) incorporated different gastrointestinal absorption rates that are commonly observed following oral administration of Co. Unice et al. (2012) included additional parameters, such as total blood volume and urinary excretion rates, to calculate Co whole blood and urine concentrations following

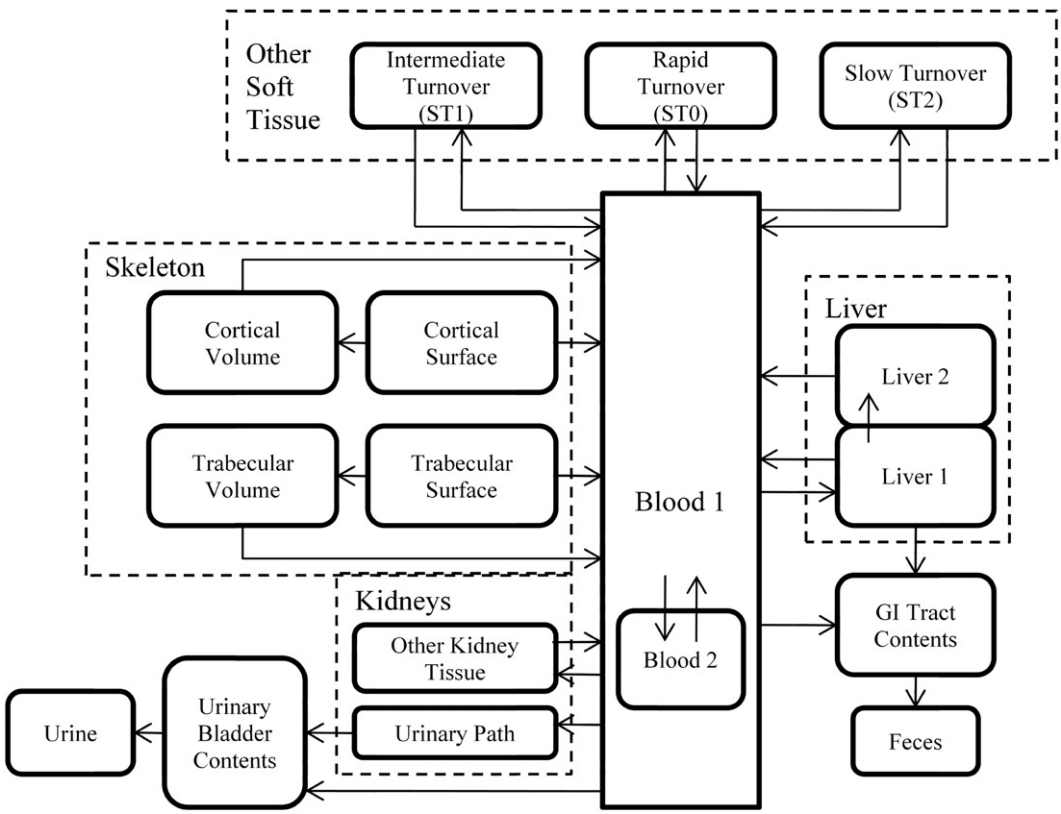

Figure 6. Systemic model for cobalt distribution in the body. Adapted from Leggett (2008).

oral administration. This model was later found to be a good predictor of blood concentrations in adult males (Tvermoes et al., 2013b).

Dose–response relationships between cobalt exposure and blood cobalt concentrations

The Unice et al. (2012) biokinetic model can be used to estimate the blood Co concentrations associated with various Co-related health effects, as well as to identify those blood Co concentrations below which effects have not been observed. As described below, these estimated blood Co concentrations can be characterized via comparisons to background Co concentrations associated with the typical diet and with respect to recently proposed guidelines for monitoring blood Co concentrations in exposed individuals.

Blood cobalt concentrations in the general population

Alimonti et al. (2005) reported that the average serum Co concentration in 110 people in Italy between the ages of 20 and 61 years was 0.19 μg/L, with 95% of individuals having concentrations below 0.41 μg/L. In populations of pre-operative joint replacement patients, and in control groups for these patients, the mean whole blood Co concentration ranged from 0.14 to 0.17 μg/L (Case, 2001; Daniel et al., 2009; Lhotka et al., 2003; Vendittoli et al., 2007). An Italian population study reported an average whole blood Co concentration of 0.39 μg/L and an average serum Co concentration of 0.21 μg/L for 375 men and 332 women (Minoia et al., 1990).

Blood cobalt concentrations associated with dietary supplementation and proposed safe doses of cobalt

Long-term Co supplementation in an amount equal to the UK guidance value of 1.4 mg/d results in predicted whole blood Co levels ranging from 7.9 to 18 μg/L after 1 year (assuming a gastrointestinal absorption rate of 15%–35%) (Unice et al., 2012). In addition, Unice et al. (2012) estimated that the use of 0.5 mg Co/d for 30 d, which is the dose recommended for hormone replacement therapy by some holistic doctors, would result in Co blood concentrations ranging between 2.5 and 5.8 μg/L. The values presented in Unice et al. (2012) are instantaneous whole blood concentrations 12 h after dosing. A summary of various exposure scenarios and estimated daily average whole blood concentrations are presented in Table 7.

Tvermoes et al. (2013b) recently reported the findings of a human study in which four healthy adult male volunteers ingested approximately 0.4 mg Co/d as $CoCl_2$ in a liquid dietary supplement for 15 or 16 d. Peak Co blood concentrations among the four adult male volunteers ranged from 1.8 to 5.1 μg/L (Figure 7). The observed blood Co concentrations were within 5% of the predictions of the Unice et al. (2012) model when assuming a gastrointestinal uptake efficiency of 15% to 35% (Tvermoes et al. 2013b). These findings indicate that the Unice et al. (2012) model is a reasonably accurate tool for estimating blood Co concentrations in males as a function of oral dose. In the discussion below, we employ the model to estimate and characterize the blood Co concentrations associated with the human effect and no effect doses in Tables 3–6 and Figures 2–5.

Table 7. Estimated daily doses of Co and corresponding Co whole blood concentrations for various exposure scenarios. Estimated average daily concentration of Co in whole blood in a 70-kg adult with 25% gastrointestinal absorption factor following oral ingestion of soluble inorganic Co for various exposure scenarios. The values in parentheses indicate the range of predicted concentrations corresponding to gastrointestinal absorption factors between 15% and 50% (Unice et al., 2012).

Exposure scenario	Daily intake (μg/d)	Estimated blood concentrations (μg/L)
Co dietary supplements marketed in the US*	200	1.8 (1.1–3.7)
	400	3.7 (2.2–7.3)
	1000	9.2 (5.5–18)
Hormone replacement therapy†	500	4.3 (2.6–8.6)
	1120	9.7 (5.8–19)
Historical Co use in beer associated with cardiomyopathy concurrent with insufficient dietary intake*	2800	26 (15–515)
	10 000	92 (55–180)
Co ingestion for increased RBC production and erythropoietin (Epo) transcription induction*	68 000	620 (370–1200)
Historical Co administration for treatment of anemia‡	11 000	88 (53–180)
	68 000	550 (330–1100)

Note, estimated average based on: *365 d of oral ingestion; †90 d of oral ingestion; or ‡30 d of oral ingestion.

Blood cobalt concentrations associated with various health effects in healthy and susceptible individuals

The human and animal exposure studies described earlier were used to identify blood Co concentrations at which hematological, thyroid, cardiovascular and neurological effects have, and have not, been reported. Where necessary, the biokinetic model was used to convert oral doses to blood Co concentrations (a GI absorption rate range of 15%–35% was used); in those few instances in which whole blood or serum Co concentrations were reported, we relied on the measured concentrations. Specific details concerning the derivation of the blood Co concentrations for these studies can be found in Finley et al. (2012a). For the model estimated human blood concentrations, animal doses were converted to human equivalent doses using $BW^{3/4}$ (body weight) scaling for subchronic and chronic dosing periods and straight BW scaling for acute single dose administration studies with severe observed effects (USEPA, 2011).

The results are summarized in Figures 2–5. Blood Co concentrations of approximately 300 μg/L and higher have been associated with certain reversible hematological and thyroid responses (polycythemia (Davis & Fields, 1958) and reduced iodide uptake (Roche & Layrisse, 1956), respectively) in humans, while higher Co whole blood and serum concentrations have been associated with a risk of more serious neurological effects in the available human studies (Bowie & Hurley, 1975; Duckham & Lee, 1976; Meecham & Humphrey, 1991). For example, in Duckham & Lee (1976) and Bowie & Hurley (1975), hearing impairment was reported in four patients with serum Co concentrations ranging between 420 and 2100 μg/L at the end of the study (average 1087 μg/L from Bowie & Hurley (1975); Figure 4); their peak Co serum concentrations ranged between 820 and 2100 μg/L. In healthy individuals (i.e., individuals not exhibiting chronic alcoholism, severe malnutrition/hypoalbuminemia, severe sepsis or kidney

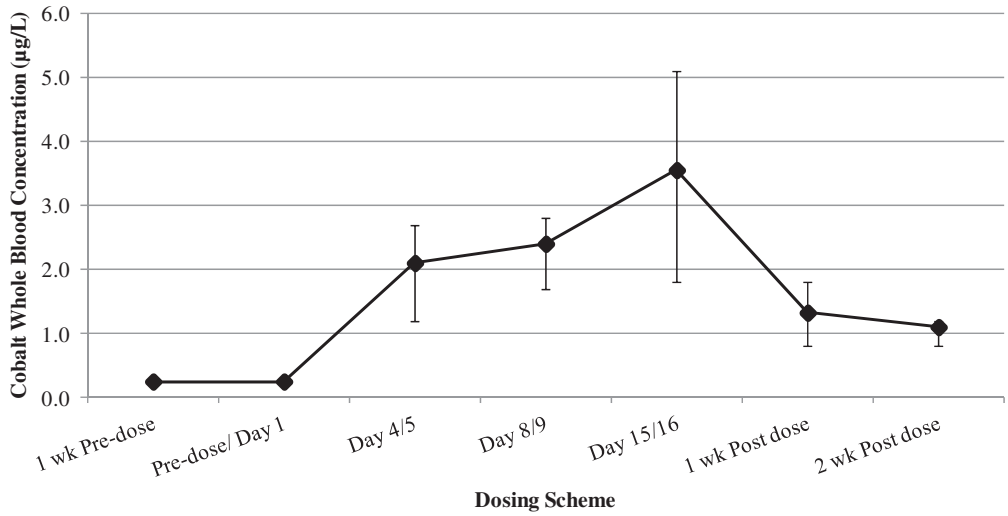

Figure 7. Time course of the observed Co whole blood measurements following dietary supplementation with 0.4 mg Co/d for 15 d. Co whole blood concentrations increased after beginning the Co dietary supplement and Co was quickly eliminated from the blood after exposure stopped as indicated by the decrease in Co whole blood concentration. All 1 week pre-dose and pre-dose/day 1 data were less than the reporting limit of <0.5 µg/L; data points for 1 week pre-dose and pre-dose/day 1 values are half the reporting limit. Black circles represent the mean Co whole blood concentration reported for a particular time point and error bars span the maximum and minimum Co whole blood concentrations reported for that time point (Tvermoes et al., 2013b). All volunteers were ChemRisk employees.

failure), blood Co concentrations below 300 µg/L have not been associated with consistent adverse responses attributable to Co intake.

As shown in Figure 5 and Table 7, cardiac effects in malnourished Co beer drinkers who were chronic, severe alcoholic beer drinkers occurred at blood Co concentrations of approximately 15–180 µg/L based on an assumed oral uptake rate of 15–50% using the Unice et al. (2012) model. This oral Co uptake estimate (and associated blood Co concentrations) may be understated because the affected beer drinkers were protein malnourished, likely triggering enhanced oral absorption of Fe and Co (Christensen et al., 1993; Elinder & Friberg, 1986; Harp & Scoular, 1952; IARC, 2006; Moshtaghie et al., 2004; Smith et al., 1972; Sorbie et al., 1971; WHO, 2006). Importantly, cardiac effects did not occur in well-nourished beer drinkers who received similar Co doses from beer (Figure 5).

Thyroid responses occurred in sickle cell children treated daily with approximately 1.4 to 1.8 mg Co/kg body weight for 5 weeks to 3 months with reported Co serum concentrations of 750 and 1950 µg/L in two of the patients (Figure 3); yet no such effects occurred in other (non-sickle cell) children treated daily with 1.8 mg Co/kg body weight for 10 weeks who had estimated blood Co concentrations of 650 µg/L (Figure 3). There are two plausible conceptual explanations for this bimodal response: (1) target organs in susceptible individuals are more sensitive to the effects of Co (i.e., the dose–response curve is shifted to the left); and/or (2) Co simply partitions to higher levels in the target organs of susceptible individuals. As described in subsequent sections, we believe that in most cases, Co susceptibility is related to unusually high partitioning of free Co ions in the target tissues due to a decreased serum protein binding of Co.

It is important to note that the biokinetic model far underpredicts blood Co concentrations in individuals with a nonfunctioning renal system who depend on renal dialysis to clear waste products; yet dialysis is not an effective treatment for clearing Co. For example, the estimated blood Co concentrations from the patients by Duckham & Lee (1976) and Bowie & Hurley (1975) are at least 300 µg Co/L lower than the measured serum values. This difference is generally more than would be expected when comparing Co serum and whole blood concentrations. Further, it is known that renal failure patients accumulate higher Co blood concentrations compared to healthy individuals administered a similar dose. For example, Curtis et al. (1976) measured Co whole blood concentrations in two hemodialysis patients and one normal subject receiving 50 mg of $CoCl_2 \cdot 6H_2O$/d for 2 weeks and found that the blood Co concentrations in the hemodialysis patients after 2 weeks of dosing were significantly higher than those measured in the normal subject: approximately 400 and 800 µg Co/L versus approximately 100 µg Co/L in the normal subject (Curtis et al., 1976). It is also important to note that Fe deficiency has been found to increase Co absorption in both animals and humans (Barceloux, 1999; Reuber et al., 1994; Sorbie et al., 1971; Valberg et al., 1969) and, indeed, findings suggest that Co and Fe may share a common intestinal uptake mechanism that is up-regulated in an Fe deficient state (Thomson et al., 1971). Accordingly, it is expected that patients who are either anemic or anephric (or both, such as in Duckham & Lee (1976) and Bowie & Hurley (1975)) have blood Co concentrations much higher than those predicted by the biokinetic model (the model assumes normal kidney clearance of Co and a relatively low GI uptake of 15%–35%). As explained further below, the susceptibility of these individuals to systemic Co toxicity depends on the Co dose and the individual's capacity for protein binding of Co.

In sum, there are rare inconsistencies in the dose–response relationships for adverse effects of Co among individuals with certain underlying disease states. However, certain generalizations are true for most healthy people at blood Co concentrations ranging up to those commonly observed with Co therapy for anemia (e.g., up to 300 µg/L). Finley et al. (2012a) reviewed the available dose–response data for

adverse effects in humans and animals at varying blood Co concentrations and concluded that ''biological responses and adverse effects in humans were not observed below measured or estimated blood Co concentrations of 300 μg/L, but were consistently observed at approximately 700–800 μg/L and higher.'' As illustrated in Figures 2 and 3, the lowest blood Co concentrations (e.g., 300 μg/L) are associated with effects in most patients or volunteers receiving Co included polycythemia (Bowie & Hurley, 1975; Davis & Fields, 1958) and reduced thyroidal iodine uptake (Jaimet & Thode, 1955; Roche & Layrisse, 1956). These less serious adverse effects were documented to be reversible upon cessation of Co exposure (Finley et al., 2012b).

The rare, more serious adverse consequences of systemic Co exposure in humans include vision or hearing impairment and peripheral neuropathy (Bowie & Hurley, 1975; Duckham & Lee, 1976; Meecham & Humphrey, 1991). These effects typically occurred at higher doses, e.g., at peak Co (serum or whole blood) concentrations ranging between 820 and 2100 μg/L or higher (Bowie & Hurley, 1975; Duckham & Lee, 1976; Meecham & Humphrey, 1991). As illustrated in Table 7, lethal cardiomyopathy has been observed at model-estimated whole blood Co concentrations of 15 to 180 μg/L (assuming 15%–50% oral absorption) in chronic, severe alcoholics with protein malnutrition and probable underlying chronic disease of the liver and heart from alcohol abuse (Kesteloot et al., 1968). Accordingly, the observation of myocardial degeneration or acute neuropathy in persons with sustained elevation of blood Co concentration at sufficiently high levels may call for evaluation of Co susceptibility related to altered Co kinetics.

Cobalt-induced adverse effects: the importance of free Co(II) and possible modes of action

Compared to unbound metals, most protein-bound metals in the blood are less bioavailable to cellular receptors and uptake mechanisms, and therefore are less likely to elicit a specific biological response. The biological responses elicited by dissolved metals are thus generally thought to be a function of their free-metal-ion-concentration. The importance of the free metal ion concept was first identified during the 1970s, when free-metal-ion-activity was demonstrated to be a better predictor of metal toxicity than the total dissolved metal concentration (Sunda & Lewis, 1978). In addition, sequestration of metals by detoxifying proteins, such as metallothioneins and glutathione-S-transferases help prevent metal toxicity. In ecological risk assessment, this concept is referred to as the biotic ligand model (BLM). The BLM assumes that, for a given situation, the bioavailability of the metal is directly related to the free-metal-ion-activity, and metal toxicity is then estimated based on the amount of free metal, metal hydroxide and competing cation activities to which the biotic ligand is exposed (Vigneault & Gopalapillai, 2009). The proposed mechanisms of Co toxicity described below are dependent upon the presence and ability of free Co(II) ions to interact with various proteins and receptors. Co(II) ions are the same as are released into the blood from persons with Co-containing implants.

Factors influencing the relative distribution of free versus bound cobalt

The equilibrium concentration of free Co(II) ions in blood involves complex ligand-binding interactions ranging from strongly-bound to weakly-bound to unbound (free) Co ions. Shifts in the blood equilibrium toward sustained higher concentrations of free Co(II) ions may be predominantly affected by the capacity of stronger binding sites in blood and tissues. Co(II) ions are the toxicologically relevant Co species. This is due to the physical/chemical properties of Co(II) ions including size, aqueous phase stability, charge and binding characteristics that allow them (but not Co(III) or Co(0)) to participate in specific receptor activation, ion channel transport, and other interactions that lead to adverse effects of excessive Co exposure.

Albumin binding

Serum albumin, lipoic acid, reduced glutathione and other strong binding sites for Co(II) ions in the blood may be a key part of the puzzle in identifying inter-individual susceptibility to systemic Co. Human serum albumin is known to contain at least four divalent cation-binding sites, and is normally capable of strongly binding between two and three Co(II) ions per molecule (Bar-Or et al., 2000, 2005; Mothes & Faller, 2007; Oettl & Stauber, 2007; Sadler et al., 1994). Initial work in this area, conducted by Nandedkar et al. (1972), identified two high-affinity Co-binding sites along with 23 sites of lower affinity. The affinity constants derived by Nandedkar et al. (1972) indicate a ratio of bound Co to free Co of approximately 11 for a nominal albumin concentration of 44 g/L in serum (Figure 8A). As noted earlier, in healthy adults, for total serum Co concentrations of 1 μg/L to 3000 μg/L, the estimated fraction of free Co in serum is expected to fall within the narrow range of 8.3% to 8.5% of total Co, respectively. Twenty percent is predicted to bind to lower-affinity binding sites on serum albumin, and 72% of Co is predicted to bind to the higher-affinity binding sites (Figure 8B). Jansen et al. (1996) confirmed a low fraction of free Co in serum ranging from 4.7% to 12% in a healthy volunteer administered ^{55}Co with sample preparation by trichloroacetic acid precipitation and dialysis methods.

A new size exclusion chromatography method reported by Kerger et al. (2013a) allows for a direct analysis of serum for ''large molecular Co'' (including albumin-bound) and ''small molecular Co'' (including free ions and <1 kDa Co-complexes). Application of this Co speciation assay to undiluted serum from five Co-containing hip implant patients led to reported findings (mean ± standard deviation) of 94.3 ± 1.3% large molecular Co species and 5.7 ± 1.3% for small molecular Co species. A validation study of this new method was conducted by Kerger et al. (2013b) for a set of 137 serum samples collected from 12 volunteers participating in a 90-d study of $CoCl_2$ supplement ingestion ~1 mg Co/d. They reported that volunteers with total Co in serum ranging up to 146 μg/L showed comparable results to the hip implant patients for Co speciation: 95.7 ± 1.6% large molecular Co and 5.5 ± 3.4% small molecular Co. This new assay was not able to separately quantify free Co(II) ion concentrations, but does demonstrate the high Co-binding capacity of human

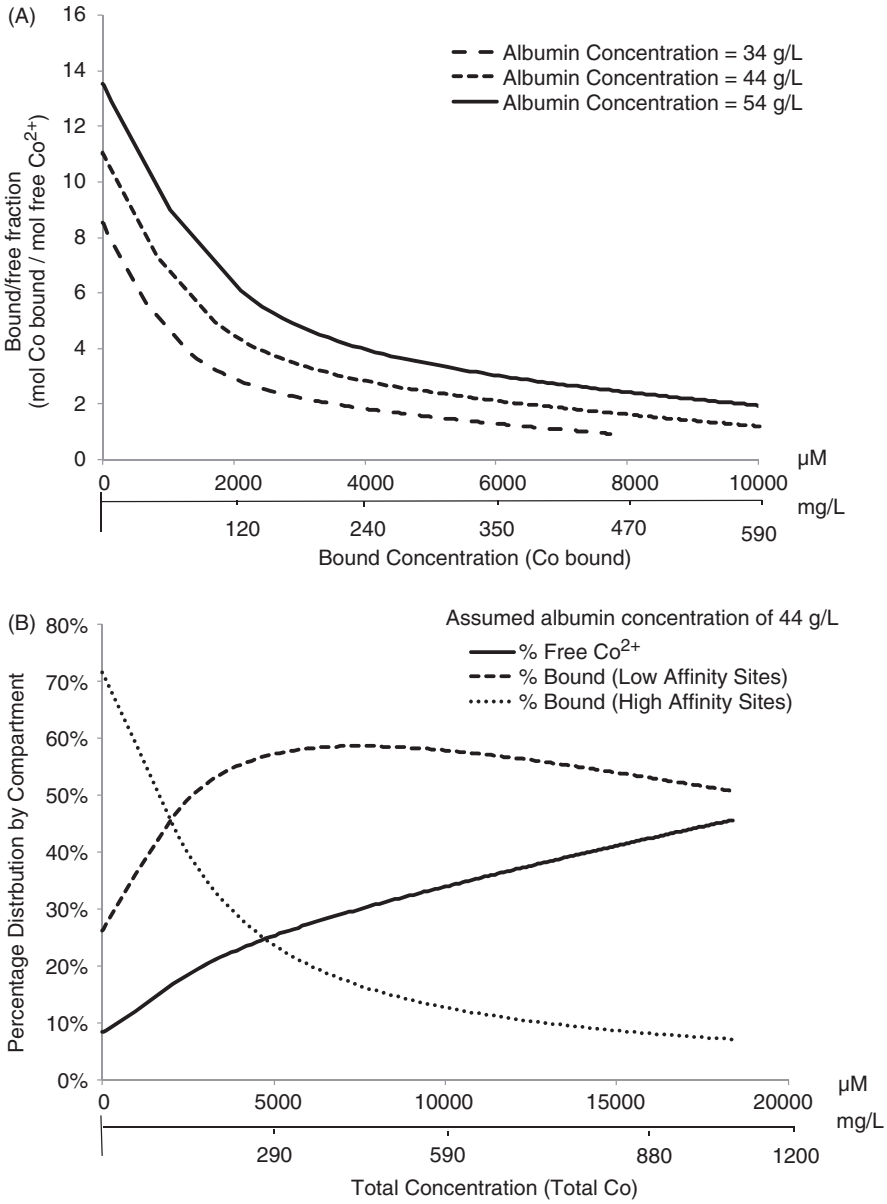

Figure 8. Plots of bound and free Co fractions based on the association constant (K_i) and number of sites (n_i) for two classes of binding sites identified from a titration experiment with human serum by Nandedkar et al. (1972), where $K_1 = 6500\,M^{-1}$, $n_1 = 2$, $K_2 = 150\,M^{-1}$ and $n_2 = 23$. (A) Fraction of bound/free Co versus bound Co for a range of typical albumin levels presented in the format of a conventional Scatchard plot. (B) Predicted percentage distribution of Co by a compartment for nominal serum albumin concentration of 44 g/L.

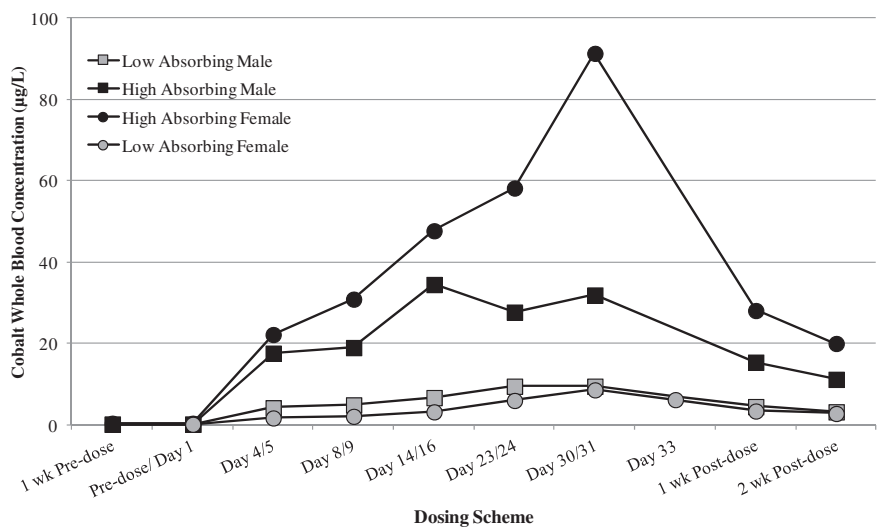

Figure 9. Time course of the observed Co whole blood measurements following dietary supplementation with ~1.0 mg Co/d for an average of 31 d for selected volunteers. Co whole blood concentrations increased after beginning the Co dietary supplement and Co was quickly eliminated from the blood after exposure stopped as indicated by the decrease in Co whole blood concentration. All 1 week pre-dose and pre-dose/day 1 data that were less than the reporting limit of 0.5 μg/L for whole blood are represented as half the reporting limit. The symbols represent the reported Co whole blood concentration for that particular time point (Tvermoes et al., 2013a).

serum albumin, and provides an objective measurement tool for identifying individuals who may have altered Co kinetics due to a lowered albumin cobalt binding (ACB) capacity. The results also appear to be reasonably aligned with earlier estimates of albumin-bound Co fractions using binding affinity (Nandedkar et al., 1972) or protein denaturation and dialysis (Jansen et al., 1996).

Several important observations can be made from an analysis of the early data on Co(II) binding characteristics generated by Nandedkar et al. (1972). First, significant changes in the fractional distribution between the low- and high-affinity binding sites can occur under the following four exceptional circumstances: (1) very high Co serum concentrations (>>3000 μg/L); (2) substantial Co displacement from high-affinity binding sites by competing species; (3) severe depletion of albumin levels or albumin damage that alters the strong binding sites; and/or (4) other events leading to the depletion of the number of stronger binding sites available. Second, albumin concentration and quality (i.e., Co(II)-binding capacity) are important determinants of the fraction of bound and free Co. Nandedkar et al. (1972) confirmed that the addition of crystalline human serum albumin to serum resulted in a proportionate increase in Co(II)-binding capacity. Third, Co(II)-binding capacity was found to be sensitive to pH. For example, with a 30 μM free Co concentration, dialysis equilibrium experiments indicated a 3.6-fold increase in the mole fraction of bound Co when the pH was increased from 6.7 to 8.6. Fourth, the association constants indicate that under normal physiological conditions (and in the absence of a competing species present in appreciable amounts), approximately 90% to 93% of total Co in serum is expected to be bound to albumin. This finding is consistent with the albumin-Co bound fraction reported by Kerger et al. (2013a,b) and emphasizes the importance of understanding the diseases or mechanisms that could potentially release free Co(II) from the protein-bound reservoir or diminish the capacity of the reservoir to sequester Co(II) ions.

To date, there is little information available to determine the magnitude of decrease in albumin levels required to sufficiently increase free Co(II) concentrations to induce clinically important effects. Kerger et al. (2013a) reported that spiking $CoCl_2$ in human serum samples at a concentration of 2500 μg Co/L resulted in ~90% distributing to the albumin-Co species. Since the albumin-binding capacity (80 to 120 mg Co/L) far exceeds the blood Co concentrations even for highly exposed persons (the highest blood and serum Co concentrations ever reported are 6500 μg/L and 2100 μg/L, respectively) (Bowie & Hurley, 1975; Zywiel et al., 2013), the reduction in blood albumin would have to be substantial to result in an increase in free Co. However, further research is required to quantitatively characterize the dose–response relationships for free Co in blood and tissues.

Displacement of cobalt from albumin

Multiple compounds present in serum can compete for the same albumin-binding sites, and may displace molecules that are already bound. A classic example in drug therapy is the displacement of bilirubin by penicillin-class antibiotics, which can result in penicillin-induced jaundice (King & Parke, 1987). Because there are multiple albumin-binding sites with varying specificities and affinities, different divalent metals will only exhibit an interaction effect when there are: (1) electrostatic interactions; (2) competition for the same site; or (3) a conformational change of the protein that affects a second binding site (Kragh-Hansen, 1981). Co appears to bind to three specific higher affinity sites on human serum albumin that also bind the divalent metals Cd and Zn (Site A/His67), Cd (Site B) and Cu and Ni (N-terminus site) (Mothes & Faller, 2007). Displacement of any given divalent metal already occupying a higher-affinity binding site on albumin may appreciably increase the free ion concentration of the displaced metal, as is the case with Co (King & Parke, 1987). Substantial shifts in the equilibrium toward greater free Co(II) would thus be expected to increase risk for adverse responses.

Several investigators have studied competitive binding interactions between Co(II) and other divalent metal species in the serum. Nandedkar et al. (1972) found that the addition of Mn^{2+} to plasma did not appreciably displace Co. Subsequently, Nandedkar et al. (1973) evaluated the effect of the divalent metal ions Co(II), Ni(II) and Zn(II) on the amount of Mn(II) bound to plasma proteins in equilibrium dialysis experiments. The affinity of human plasma proteins for these divalent metals was Zn > Ni > Co > Mn. The Mn–albumin complex released the metal ion after ammonium sulfate fractionation or acid treatment, in contrast to the Co-albumin complex, which was not released (Nandedkar et al., 1973). For the data corresponding to equimolar amounts of Mn(II) and each of the three divalent competitive species evaluated, an appreciable increase in the free Mn(II) with increasing affinity for albumin of the second ligand was observed. Moshtaghie et al. (2004) found that 225 μM Co(III)-citrate reduced binding of Fe to human apo-transferrin by about 20%. Fibrin stabilizing factor (Factor XIII) is an enzyme of the blood clotting process active in the presence of Ca(II), but with evidence that other divalent metals, including Ni(II), Mn(II) and Co(II), can substitute for the primary cofactor (Lewis et al., 1978). Yang and Black (1994) showed that adsorption of Co(II) to murine serum was enhanced by an equimolar addition of $CrCl_3$ and reduced by the equimolar addition of $NiCl_2$.

To our knowledge, there is no evidence to indicate that Co can be displaced from human albumin *in vivo* by other metals. Kerger et al. (2013a) reported that Co(II) from a 5 μg/L Co(II) spike in serum was not displaced from albumin-Co species at competing concentrations of 10, 100 and 1000 μg/L (as the metal chloride) for the divalent metals Fe(II), Zn(II), Mn(II), Cd(II), Ni(II) and Pb(II). Given the fact that Co has a relatively high affinity for certain divalent cation-binding sites in human albumin, significant displacement is unlikely to occur unless blood concentrations of competing metals are greater than 1000 μg/L.

Reduced albumin binding of cobalt due to polymorphisms or severe ischemia

The N-terminal portion of human serum albumin (HSA) (N-asp-Ala-His-Lys-) binds Cu, Ni and Co ions with high affinity, whereas Au, Ag and Hg ions bind cysteine-34. HSA is also a major Zn-binding protein in the plasma, although

Table 8. Various proposed mechanisms through which IMA is formed which, in turn, could allow for a much greater percentage of free Co(II) to exist in the blood (Bar-Or et al., 2008; Chen et al., 2011; Cichota et al., 2008).

Ischemia
Hypoxia
Acidosis
Free radical formation/oxidative stress
Membrane energy-dependent sodium and calcium pump disruptions
Elevated free iron and copper ion exposure

Table 9. Disease states associated with IMA or decreased albumin levels (Borderie et al., 2004; Duarte et al., 2009; Krantz et al., 2005; Sbarouni et al., 2011).

Pathologies associated with increased IMA
Cerebrovascular-ischemic stroke, subarachnoid and intracranial hemorrhage, myocardial ischemia
Peripheral vascular disease
End-stage renal disease
Advanced liver cirrhosis
Acute infections
Malignancies
Systemic sclerosis
Hypercholesterolemia
Prostatic diseases – hyperplasia or cancer
Pathologies associated with decreased albumin synthesis
Chronic malnutrition
Hypergammaglobulinemia
Stress secondary to infection, surgery, radiation, trauma
Cracinoma
Cirrhosis
Hypothyroidism
Hepatic toxins
Pathologies associated with increased albumin loss
Nephrotic syndrome
Protein-losing enteropathy
Severe burns
Hypermetabolic states – Cushing's syndrome, hyperthyroidism

there is some debate as to the nature and location of this site (Quinlan et al., 2005). One analog of human albumin has an altered N-terminus configuration that reduces the binding capacity in some people (Bar-Or et al., 2001); this change occurs in genetic variants of low incidence (~0.1%). To our knowledge, no physiological risk factors for this rare genetic variant (gender, race, etc.) have been identified. Further, it is unknown whether individuals with this genetic defect truly experience a significant increase in free Co at Co doses relevant to humans.

In addition to genetic polymorphisms that may lower albumin-binding capacity for Co, considerable research has demonstrated that the N-terminus of serum albumin may become damaged as a result of oxidative stress, ischemia, hypoxia and acidosis (Hausen et al., 2012). Modifications to the N-terminus of albumin results in the formation of ischemia-modified albumin (IMA); the precise mechanism for IMA generation is not well understood, but it is thought that ROS produced during ischemic events may result in site-specific modifications to the N-terminus, which in turn disrupts Co binding (Bar-Or et al., 2000). A summary of proposed mechanisms for IMA generation is presented in Table 8. Pathologies associated with increases in IMA levels include certain acute and chronic disease states involving bursts of strong inflammatory responses as seen with ischemia following a heart attack. Elevated concentrations of free radicals that are generated during severe episodes of ischemic damage, hemorrhage and/or inflammation can result in the partial denaturation of albumin proteins and increased free metal ion levels (Awadallah et al., 2012; Davies & Delsignore, 1987; Marx & Chevion, 1985; Oettl & Stauber, 2007).

This characteristic was the basis for the clinical test for IMA known as the ACB assay that was approved by the Food and Drug Administration for clinical use for detecting recent cardiac ischemia (Bar-Or et al., 2000, 2001, 2005; Bhagavan et al., 2003; Mothes & Faller, 2007). The ACB test involves adding a known quantity of Co(II) ions as $CoCl_2$ to an aliquot of human serum to allow for saturation binding of albumin; dithiothreitol (DTT) is then added to capture the remaining free Co(II). The DTT-Co complex is then measured by spectrophotometry in order to gauge the degree of IMA present in the serum sample; more DTT-Co corresponds to greater IMA. While the ACB test is useful for measuring IMA, many different disease states or inflammatory conditions other than cardiac ischemia were later discovered to lead to elevated IMA (Govender et al., 2008; Lippi et al., 2005). Thus, the ACB test results were not highly specific to cardiac ischemic events. The assay is no longer available for diagnostic purposes in the US.

Though not highly specific to cardiac ischemia, IMA is regarded as a biomarker of oxidative stress related to ischemia-reperfusion in different clinical conditions associated with oxidative stress, such as type II diabetes, chronic kidney disease, hypercholesterolemia and systemic sclerosis. As such, the ACB test has identified a series of inflammatory disease states that increase IMA and thus inherently reduce the normal Co(II) ion-binding capacity of human serum albumin (Table 9). For example, Kaefer et al. (2010) reported higher levels of IMA in patients with type II diabetes (i.e., 0.535 ± 0.125 ABSU compared to 0.411 ± 0.086 ABSU in control subjects) (Kaefer et al., 2010). The reported IMA value of the patient described by Catalani et al. (2011) was just above 0.5 ABSU, and, according to Bar-Or, an ABSU value greater than 0.4 indicates a reduced Co-binding capacity (Bar-Or et al., 2000; Catalani et al., 2012).

Increased IMA has also been reported using the ACB test for patients with B-thalassemia (Awadallah et al., 2012), systemic sclerosis (Borderie et al., 2004), cirrhosis (Chen et al., 2011) and hypercholesterolemia (Duarte et al., 2009). The presence of a disease or inflammatory state alone is probably insufficient information to judge possible susceptibility to Co toxicity. In principle, with more acute and severe inflammatory responses greater amounts of IMA are generated, which could potentially result in sustained periods with increased free Co(II) ions, and (generally reversible) adverse health effects with sufficient free ion systemic dose and exposure duration. IMA is reported to be cleared from the blood relatively rapidly, with a half life of about 6–12 h, whereas the mean half-life of albumin is about 20 d (Bito et al., 2005; Borderie et al., 2004; Oettl & Stauber, 2007). Sufficient accumulation of IMA in some individuals may cause major shifts in Co(II) ion-binding capacity in blood that

is sustained until liver albumin production returns the blood to the normal albumin range of 3.4–5.4 g/dL. Consequently, the confluence of disease states resulting in high accumulation of IMA in conjunction with hypoalbuminemia might be expected to enhance susceptibility to increasing blood concentrations of free Co(II) ions and increase risk of adverse health effects.

We are unaware of any data indicating the minimum concentration of IMA required to cause a significant increase in free Co ion concentrations in Co-exposed humans.

Molecular mechanisms of action of free Co(II)

The toxicity of Co is thought to occur through various mechanisms that specifically depend on free Co(II) ion interactions with cellular receptors, ion channels and biomolecules. Normally, the valence state of the free Co in blood will be Co(II). Depending on dose, the following mechanisms may be involved in Co toxicity: (1) generation of reactive oxygen species and lipid peroxidation; (2) interruption of mitochondrial function; (3) alteration of Ca and Fe homeostasis; (4) interactions with body feedback systems triggering erythropoiesis; (5) interruption of thyroid iodine uptake; and (6) induction of genotoxic effects and possible perturbation of DNA repair processes. Where possible, we identify the blood or tissue Co concentrations at which these mechanisms may begin to induce significant adverse effects. Interestingly, it has been suggested that a tolerance to Co may be established following initial low-dose exposures (Stokinger, 1962).

Fenton-like reaction to generate reactive oxygen species

It has been reported that certain Co(II) complexes may facilitate the formation of free radical species from hydrogen peroxide (H_2O_2) *in vitro* and *in vivo. In vitro*, electron spin trapping studies have reported that a mixture of 1 mM Co(II), 2 mM H_2O_2 and 2 mM anserine in a phosphate buffer (pH 7.4) solution can catalyze the generation of free radicals from H_2O_2 via a Fenton-type reaction (* indicates free radical species) (Mao et al., 1996).

$$Co(II) + H_2O_2 \rightarrow Co(III) + {}^{*}OH + OH^{-}$$

Notably, the millimolar concentrations of Co(II) and hydrogen peroxide utilized to study these reactions are not likely to be observed *in vivo* except perhaps within lysosomes. It is also important to note that *in vitro* electron spin trapping studies have reported that Co(II) alone does not efficiently generate hydroxyl radicals (*OH) from H_2O_2, but chelation of Co(II) with certain agents, such as glutathione and anserine, changes the oxidation potential of Co(II), and can enhance the formation of *OH from H_2O_2 (Hanna et al., 1992; Leonard et al., 1998; Shi et al., 1993). Omission of any one component sharply reduces the amount of *OH radical generation, indicating that chelators, such as anserine, modulate the oxidation potential of Co(II), and this modulation appears to be specific to di-oxygen bridge compounds like the artificial lipid peroxide compounds that these authors studied (Hanna et al., 1992; Mao et al., 1996). However, the toxicological relevance of these reactions is unclear due to the high concentrations of Co(II) and hydrogen peroxide required for these reactions.

Leonard et al. (1998) have also investigated the possibility of free radical generation by Co metal particles (size 0.1–1.5 μm). These authors reported that electron spin trapping measurements provided evidence that high concentrations of these fine Co metal particles (10 mg/mL) in aqueous solution can react with dissolved oxygen to generate *OH radicals in the presence of superoxide dismutase, but no such reaction was observed in the absence of superoxide dismutase. Based on electron spin trapping evidence of *OH formation, the authors proposed the following reaction equation:

$$Co(I) + H_2O_2 \rightarrow Co(II) + {}^{*}OH + OH^{+}$$

Soluble and insoluble Co compounds may be subjected to Fenton-type reactions within lysosomal compartments of macrophages as a result of the body's defense mechanisms for eliminating foreign particles. For example, macrophage cells can engulf insoluble Co particles and utilize hydrogen peroxide and other lysosomal components to attempt to dissolve the foreign particle; under some conditions, these Fenton-type reactions may lead to ROS production and lipid peroxidation that may functionally damage and/or lyse the macrophage. Further, ROS could result in protein oxidation, potentially leading to protein damage and denaturation, as seen in some *in vitro* macrophage assays at concentrations ranging from 100 to 170 μM Co (Caicedo et al., 2009; Petit et al., 2005).

With respect to chemical properties, however, Co(II) is poorly reactive, meaning it does not generate ROS on its own; it apparently requires chelation with certain organic ligands and the presence of H_2O_2 and Co(II) at millimolar concentrations that may only occur within lysosomes. In addition, the quantity of free radicals generated has been reported to depend on the chemical structure of the ligands present, and the reactivity of Co(II) under certain conditions was not always reported to be enhanced by the same ligands (Shi et al., 1993). Further, the relevance of the tested combinations of chelators and ligands remains to be demonstrated *in vivo* and *in vivo* Co(II) ions can bind to a number of proteins, such as albumin or other natural ligands, reducing their bioavailability and subsequent reactivity, which *in vitro* studies cannot account for. Therefore it is difficult to predict the ability of Co(II) to mediate oxidative damage *in vivo* based on the results of available *in vitro* studies.

Some animal studies have reported ROS generation, lipid peroxidation and other associated effects at relatively high doses (i.e., maximum tolerated dose not causing acute lethality). Kasprzak et al. (1994) reported that a single intraperitoneal administration of Co(II) as Co acetate (3 mg or 6 mg/kg body weight) produced oxidative DNA damage in the kidney, liver and lung in rats sacrificed 2 and 10 d after dosing. Mathur et al. (2011) reported a significant increase in hepatic lipid peroxidation and irregularly shaped hepatic lobules in rats orally exposed to 25 mg Co/kg-d as $CoCl_2 \cdot 6H_2O$ for 60 d.

It has been reported that single subcutaneous injections in rats (27 mg Co/kg) and guinea pigs (36 mg Co/kg) resulted in a significant increase in lipid peroxidation in the liver, as well as changes in glutathione and hepatic levels of superoxide dismutase, catalase, heme oxygenase and glutathione

peroxidase after an exposure for 24 h (Christova et al., 2002). In addition, Christova et al. (2001) reported that repeated subcutaneous injections of smaller doses (4.5 to 13.5 for a total of 27 mg Co/kg-d) of $CoCl_2$ in rats resulted in increased levels of hepatic lipid peroxidation as well as altered levels of certain antioxidant enzymes and glutathione. Based on the kinetic model of Unice et al. (2012), estimated blood Co concentrations associated with ROS/lipid peroxidation in the above studies exceed 1900 μg/L. Such high concentrations have rarely been reported in humans and thus are of questionable relevance to chronic human exposures. In addition, it is highly likely that directly injected bolus doses overwhelm the serum albumin-binding capacity for a period of time, thus permitting free Co ion to accumulate in tissues (and cause oxidative damage) at levels that would not be otherwise attainable. This supposition is consistent with the fact that Singh et al. (2010b) found no evidence of increased ROS activity in cardiac tissue of rats orally exposed to 12.5 mg Co/kg for 7 d, equivalent to a whole blood concentration of 810–1900 μg Co/L in humans.

In summary, the tissue concentrations of free Co ion required to induce ROS activity and lipid peroxidation have not been evaluated in detail, and may be tissue-specific. It is important to keep in mind that the human body possesses numerous defense mechanisms designed to keep oxidative damage in check that may neutralize ROS and/or bind Co(II) ions in the blood or within cells. These defense mechanisms, such as binding with serum albumin or amino acids, can effectively avert Fenton-type reactions and associated lipid peroxidation in the absence of ionic Co doses exceeding the binding capacity of these redundant and regenerable oxidative damage defense systems. Thus, while Fenton-like reactions of Co(II) and lipid peroxidation may play a key role in Co toxicity, these reactions appear to be largely limited to extremely high dose conditions and/or occur within lysosomes that may sequester ROS and limit the damage to other cells or tissues.

Interactions with cellular respiration and mitochondrial function

The mitochondria appear to be a principle target of Co toxicity based on observations of decreased mitochondrial membrane potential and dose-dependent ATP depletion by Co(II) ions at relatively high concentrations *in vitro* (Karovic et al., 2007). Co(II) ions at high concentrations may depress mitochondrial oxygen uptake by complexing with sulfhydryl (–SH) biomolecules that are co-factors for cellular respiration in the citric acid cycle. Co(II) ions have been shown to have a high affinity for sulfur atoms and readily bind to sulfhydryl-containing compounds, hindering the function of certain enzymes. For example, lipoic acid is a fatty acid biomolecule with vicinal sulfur atoms that act as an important antioxidant and co-factor in human mitochondria. Excessive free Co(II) can consume lipoic acid stores within mitochondria and interrupt the citric acid cycle by preventing reactions that require lipoic acid as a co-factor (e.g., oxidative decarboxylation of pyruvate to acetyl coenzyme A and α-ketoglutarate to succinyl CoA) (Alexander, 1972; Baskin & Behonick, 2000; Webb, 1962, 1964; WHO, 2006). Severe disruption of mitochondrial function is thought to trigger altered Ca^{2+} signaling and increased generation of oxygen radicals, and produce pro-apoptotic factors that lead to cell death (Karovic et al., 2007). Consequently, the extent of damage to key organs with high energy demand (e.g., the heart) from this mitochondrial interruption is likely controlled by the sustained presence of higher free Co(II) ion concentrations in the blood and tissues.

Clyne et al. (1990) orally dosed rodents with 4.2 or 8.4 mg Co/kg-d for 8 weeks and reported no general inhibition of myocardial mitochondria ATP-production rate at either exposure concentration despite reporting a 70-fold increase in myocardial mitochondria Co concentration at the highest exposure concentration. The predicted human blood Co concentration associated with the highest oral dose (8.4 mg Co/kg body weight) ranges from 710 to 1700 μg/L. In contrast, Wiberg et al. (Wiberg et al., 1967; Wiberg, 1968) reported a reduction in myocardial mitochondria respiration in rats treated with 4 mg Co/kg-d for 8 d. However, these rats were exposed via intraperitoneal injection and this dose is associated with a maximum predicted 24 h average human blood concentration of 1900 μg/L which, as noted above, may have largely existed as free Co that quickly equilibrated into tissues. Thus, based on the available animal data, it is difficult to assess the tissue concentration of free Co ions that would impair cellular respiration and mitochondrial function. As described in detail later, it is likely that this mechanism played some role in the cardiomyopathy in beer drinkers, who were estimated to have blood Co concentrations of 15–180 μg/L (Table 7). However, it is also clear that other factors contributed significantly to the cardiomyopathy, and hence the 15–180 μg Co/L range of values should not be interpreted as causal for this adverse effect.

Interactions with calcium and iron homeostasis

As noted earlier, the toxicity of Co occurs in part from its ability to compete for divalent cation-binding sites in the body with other essential trace elements. For instance, Co(II) ions may compete with other divalent metals such as Zn, Fe, Mn, and Mg for absorption from the gastrointestinal tract, which may result in vitamin and Fe deficiencies (Domingo, 1989; Flanagan et al., 1980; Thomson et al., 1971). As noted earlier, the physical/chemical similarity of Co(II) and Ni(II) provides the substitution of these metals for Fe(II) in certain enzymes and transporter proteins. For example, the non-heme metal-binding site in phthalate dioxygenase requires Fe(II) to successfully hydroxylate and activate the enzyme, but several other divalent metal ions (e.g., Cu, Co, Mn, Ni or Zn) can populate that metal site and block the hydroxylation/activation (Maxwell & Salnikow, 2004). Co(II) and Ni(II) ions are reported to bind more tightly to DMT-1, which is partly responsible for Fe(II) transportation into cells; thus, sustained increases in circulating blood concentrations of Co(II) and/or Ni(II) could lead to a depletion of cellular Fe concentrations (Maxwell & Salnikow, 2004). Such cellular Fe depletion does not last long, however, since biofeedback mechanisms detecting low cellular Fe concentrations and low oxygenation will up-regulate the production of Fe-binding proteins to restore the balance (e.g., ceruloplasmin, transferring receptors

and other proteins involved in Fe uptake from the gut and transport into cells) (Maxwell & Salnikow, 2004).

In addition, the alteration of Ca^{2+} homeostasis achieved by blocking Ca channels is another potential action of Co(II) ions that has been associated with impairment of both steroidogenesis and neuromuscular transmission (EFSA, 2009; Weakly, 1973; WHO, 2006). For example, Weakly et al. (1973) reported that Co, in concentrations of 0.05–2.0 mM (~3000–118 000 μg/L) blocked neuromuscular transmission in isolated frog sartorius muscle preparations. Simonsen et al. (2011a,b) reported that Co(II) can compete with Ca(II) for binding sites on carrier proteins responsible for maintaining Ca^{2+} balance in RBCs, lowering intercellular Ca^{2+} and possibly affecting Ca^{2+} signaling; they note that Co(II) binding to cytosolic proteins was essentially irreversible. Co has also been reported to substitute for Zn in the Zn finger domain of certain DNA repair proteins (Kopera et al., 2004). Studies have found that Co(II) ions can bind to Ca^{2+} ATPase in the sarcoplasmic reticulum, and *in vitro* studies have shown that Co(II) ions can block Ca influx through voltage-dependent Ca^{2+} channels (Clyne et al., 1990; Diaz et al., 2005; Persson et al., 1992; Ranquet et al., 2007). Further, Co has been reported to block Ca^{2+} channels in squid axons (Baker et al., 1973) and it has been reported that Ca^{2+} channels in rat brain cells (melanotrophs) are permeable to Co (Shibuya & Douglas, 1992).

Co and Fe share at least one transport mechanism within the small intestine, such that both Co and Fe uptake are diminished when co-administered (Sorbie et al., 1971; Thomson et al., 1971). For example, Valberg et al. (1969) reported that Fe absorption was decreased in human volunteers when carrier Co was administered with Fe and a similar decrease was observed in Co absorption when carrier Fe was administered with Co. Further, Valberg et al. (1969) and Sorbie et al. (1971) reported that Co absorption was elevated in patients with certain Fe deficiencies, and, more recently, Meltzer et al. (2010) reported that, in a study of female volunteers, low Fe stores were related to higher blood Co concentrations.

In *Escherichia coli*, Co has been reported to compete with Fe for binding in scaffolding proteins required for the biosynthesis of Fe–S clusters. Fe–S clusters are inorganic cofactors required for a number of biological processes, such as respiration, DNA repair and tRNA modification (Barras & Fontecave, 2011; Fantino et al., 2010). As a result, the production of [Fe–S]-containing enzymes dramatically decreases in the presence of free Co(II) ions (Barras & Fontecave, 2011; Ranquet et al., 2007). Further, a recent study in yeast reported that Co inhibited a specific set of Fe–S dehydratase enzymes of the mitochondria (Gleason et al., 2011). In addition, Co has been reported to substitute for Fe in certain cytochromes, resulting in the inactivation of membrane-bound cytochromes in *E. coli* (Majtan et al., 2011). Co has also been reported to decrease the synthesis of cytochrome P_{450} in mice exposed to 18 mg Co/kg as $CoCl_2$ via subcutaneous injection for 1 or 2 d (Legrum et al., 1979).

While numerous lines of evidence indicate that Co can compete with Fe uptake from the GI tract and can also substitute for Fe in numerous critical cell functions, to date, the oral Co doses required to significantly reduce normal or therapeutic Fe intake and the blood/tissue concentrations required to hamper normal cell functions have not been clearly delineated.

Interactions with proteins triggering erythropoiesis

Co(II) ions at sufficient blood concentrations promote a hypoxia-like response that enhances erythropoiesis and blood vessel formation (angiogenesis). In rats orally administered $CoCl_2$ (12.5 mg Co/kg-d) for 7 d, significant increases in hemoglobin, hematocrit and RBC counts were measured in the Co supplemented group relative to the controls (Shrivastava et al., 2010). A similar dosing regimen was reported by Singh et al. (2010a) to increase hypoxia-inducible factor (HIF-1α) and related increases in metallothionein (MT-1) and hemeoxygenase (HO-1) activity, erythropoietin, vascular endothelial growth factor and glucose transporter (Glut-1). This dosing regimen (12.5 mg Co/kg-d × 7 d) did not significantly increase various indicators of oxidative stress, such as glutathione peroxidase, glutathione-S-transferase or superoxide dismutase activity (Singh et al., 2010a). Erythropoietin gene expression is stimulated by HIF-1α, which induces the expression of genes that allow for cell survival during times of low oxygen (Karovic et al., 2007). Studies have shown that Co binds to and stabilizes cytosolic HIF-1α by blocking ubiquitination and proteasomal degradation under conditions in which it would normally be degraded, such as when sufficient oxygen is present (Haase, 2010; Yuan et al., 2003).

The complex cascade of events surrounding the hypoxia-like effects of Co(II) (and Ni(II)) have been reviewed elsewhere (Ke & Costa, 2006; Maxwell & Salnikow, 2004; Simonsen et al., 2012), but deserve a brief overview here. As noted earlier, common physical/chemical characteristics of Ni(II) and Co(II) ions intertwine their biological activity, with HIF functionality being at the center of many cascades involved in Fe and oxygen homeostasis in the human body (Maxwell & Salnikow, 2004). Fe does not have a hypoxia-inducing effect, while both Ni and Co ions are capable of stimulating hypoxia-like responses through HIF stabilization. Co(II) and Ni(II) may substitute for Fe(II) in non-heme binding sites in dioxygenase enzymes that participate in the feedback mechanisms for maintaining adequate oxygenation in cells throughout the body. Such substitution of Fe(II) prevents these dioxygenase enzymes from becoming activated, which leads to an up-regulation of HIF and increased production of biomolecules to increase Fe levels, and restores adequate oxygenation, including increased release of erythropoietin. As noted earlier, Co(II) and Ni(II) may also affect HIF activity by binding to Fe(II) transporter proteins and reducing intracellular Fe(II), leading to reduced dioxygenase enzyme activity and the up-regulation of HIF. At higher doses of Co(II) that cause appreciable ROS generation, an indirect effect on dioxygenase activity and associated enzyme cascades may also affect HIF up-regulation through depletion of antioxidant/sulfhydryl compounds in cells (e.g., ascorbate, lipoic acid and glutathione) (Maxwell & Salnikow, 2004).

While the specific blood Co concentrations required to induce these mechanistic cascades is not known, the blood Co concentrations at which RBCs begin to increase is starting

to be understood. As summarized in Figure 2, increased erythrocyte concentrations in humans have been consistently observed at Co blood concentrations of 300 μg Co/L and higher, and indeed, polycythemia was a desired response from Co therapy.

Interruption of thyroidal iodine uptake

The main function of the thyroid gland is to synthesize the thyroid hormones, thyroxine (T4) and triiodothyronine (T3). Thyroid hormone production therefore requires an uptake of iodine, which is subsequently combined with the amino acid tyrosine (Patrick, 2008; Rousset, 2007). The production of T4 and T3 is regulated by thyroid-stimulating hormone (TSH), which, in turn, is regulated by auto-feedback receptors that detect T4 and T3 levels. This system forms a negative feedback loop in which TSH production is suppressed when thyroid hormone levels are sufficiently high (Bianco & Kim, 2006; Szkudlinski et al., 2002). Hypothyroidism results from insufficient production of T3 and T4. When thyroid hormones are low, TSH stimulates the growth of the thyroid as a means of increasing T3 and T4 production. However, when hypothyroidism is caused by iodine insufficiency, the thyroid is unable to produce T3 and T4, and as a result, the thyroid continues to grow and a goiter is formed.

Studies have reported that Co(II) ions inhibit iodine uptake by the thyroid through an undefined mechanism; it is possible that Co may inhibit thyroidal iodine uptake by binding to enzymes or co-factors necessary for combining iodide and tyrosine in the thyroid gland (Bucher et al., 1990; Sederholm et al., 1968). Studies have shown that thyroidal I^{131} uptake is reduced following the oral administration of Co, and that sharp rises in iodine accumulation occur after cessation of Co exposure (Kriss et al., 1955; Roche & Layrisse, 1956). The relatively rare goiter response seen in some anemic patients undergoing Co therapy is typically reversible upon ceasing therapy. Sederholm et al. (1968), for example, described an enlarged thyroid gland in a 12-year-old anephric patient undergoing Co therapy for 4 months. Co therapy was stopped, and daily treatment with 55 μg of T4 was begun; within 3 weeks of stopping Co therapy, the thyroid gland returned to normal size (Sederholm et al., 1968). Further, Kriss et al. (1955) reported that 10 mM $CoCl_2$ completely inhibited the iodination of tyrosine by the enzyme tyrosine iodinase. It should also be noted that chronic hypothyroidism from high Co intake or from other causes, if it remains untreated, can lead to optic and auditory neuropathy (Rubin, 2012; Schirrmacher, 1967).

In addition, other studies have suggested that Co may alter thyroid hormone levels via metabolic alterations affecting T4/T3 ratios. Prescott et al. (1992), for example, studied the effects of occupational Co exposure on thyroid hormone metabolism in 61 female plate painters exposed to Co blue dyes in two porcelain factories. The authors reported that occupational exposure to semisoluble Co as Co–Zn silicate ($n = 25$) did not inhibit the thyroid function, but rather increased the ratio of T4/T3, with higher T4 levels causing auto-feedback loops to inhibit further thyroid hormone production. Exposure concentrations were not reported, but it was noted that the mean urinary Co concentration in the exposed group was 1.17 μg Co/mmol creatinine, as compared to 0.20 μg Co/mmol creatinine in the control group. Similarly, an earlier study by Anbar & Inbar (1964) reported that T4 concentrations in the blood of mice following Co exposure were ''rather high,'' suggesting that Co may have shifted metabolism towards higher proportions of T4.

As with polycythemia, the blood or tissue Co concentrations required to induce the mechanistic events that ultimately result in thyroid dysfunction may not be currently understood, but the blood Co concentrations associated with clinical effects (reduced iodine uptake and the appearance of goiter) are well-defined. As summarized in Figure 3, thyroid dysfunction has been consistently observed at estimated or measured blood Co concentrations of 300 μg/L and higher.

Genotoxicity and inhibition of DNA repair

Genome stability is essential for proper cell function and survival. *In vitro* studies have suggested that the two major mechanisms involved in the genotoxic and carcinogenic potential of Co are (i) the generation of reactive oxygen species through a Fenton-like mechanism; and (ii) the inhibition of DNA repair mechanisms (Beyersmann & Hartwig, 2008). Co compounds were reported to be mostly non-mutagenic in bacterial test systems, with few exceptions in some tester strains (NTP, 1991; Ogawa et al., 1986). In contrast to the results seen in bacteria, soluble Co compounds were found to be genotoxic in certain mammalian assay systems.

In vitro studies have reported that Co(II) compounds at sufficient concentrations in certain test systems can result in DNA single strand breaks, DNA-protein cross-linkage and sister-chromatid exchanges (Baldwin et al., 2004; Beyersmann & Hartwig, 1992; Lloyd et al., 1998). *In vitro* studies have also reported that Co metal and hard metal (tungsten-carbide) at sufficiently high doses can produce DNA strand breaks, and that this DNA damage could be partially blocked by ROS scavenging (Anard et al., 1997; De Boeck et al., 1998; Van Goethem et al., 1997). These studies also noted that hard metal exposure above certain concentrations resulted in significantly more DNA strand breaks when compared to Co metal particles alone (Anard et al., 1997; De Boeck et al., 1998).

Using *in vitro* assays, above certain concentrations, $CoCl_2$ has been reported to inhibit DNA repair systems (such as nucleotide excision repair (NER)) by inhibiting the incision and polymerization step (Hartwig et al., 1991; Kasten et al., 1997). $CoCl_2$ has also been reported to modulate NER by interfering with the DNA-binding ability of the xeroderma pigmentosum A (XPA) protein via substitution of the Zn ion by a Co ion (Asmuss et al., 2000; De Boeck et al., 1998; Hartwig et al., 1991; Kasten et al., 1997). The XPA protein is required for the recognition of DNA lesions, and is critical for recruiting other NER proteins to repair damaged sites (Asmuss et al., 2000; Kopera et al., 2004; Lison et al., 2001). Co(II) compounds have also been reported to modulate the DNA-binding activity of the tumor suppressor gene p53 (Asmuss et al., 2000; Kasten et al., 1997; Palecek et al., 1999). Metallic Co has also been reported to inhibit DNA repair processes *in vitro*, although the implications of this

finding *in vivo* have not been confirmed (Beyersmann & Hartwig, 1992; De Boeck et al., 1998).

In vivo studies in hamsters, rats and mice have shown that exposure to Co(II) at relatively high doses can lead to chromosomal aberrations, micronuclei and oxidative DNA damage. For example, Palit et al. (1991) reported that the frequency of chromosome aberrations in bone marrow cells increased proportionally with dose after single oral administrations of various $CoCl_2 \cdot 6H_2O$ concentrations (~20, 10 and 5 mg Co/kg) for 6, 12, 18 or 24 h of exposure. Farah et al. (1983) treated hamsters with a total of 400 mg $CoCl_2$/kg (~182 mg Co/kg) via intraperitoneal injection over a course of 7 d and reported a significant increase in the frequency of chromosome aberrations in bone marrow and germ cells. Similarly, Suzuki et al. (1993) reported an increase in the frequency of micronucleated polychromic erythrocytes (MPCE) after injecting (intraperitoneal) mice once with 50 or 90 mg Co/kg (no such effects were observed at 25 mg/kg). Notably, these *in vivo* genotoxicity studies involved Co administration at or near lethal dosages for the studied species.

The wear debris from some hip and knee replacements has been evaluated to assess its capacity to have mutagenic activity in *in vitro* systems. For example, human cells in culture using the micronucleus assay and fluorescent *in situ* hybridization were examined by Daley (2004). The extracted-wear debris increased the concentration of micronuclei in a linear dose-dependent manner, but it was unclear if Co or Cr was the responsible agent. The authors note that ''[i]t is important to emphasize that a tissue-culture system is not an exact model of what happens *in vivo*'' (Daley et al., 2004).

In summary, the available genotoxicity studies suggest micronuclei formation and chromosome aberrations are observed in animals at Co doses sufficiently high to cause frank toxicity, but the clinical significance of these occurrences at plausible chronic human doses is not known. Similar to the ROS/lipid peroxidation mechanism of action, the relevance of these observed genotoxic effects to lower Co doses likely depends on the complex human pharmacokinetics and pharmacodynamics of Co(II) ions. The protective influence offered by the high Co(II)-binding capacity of serum albumin and other biomolecules may preclude any important consequences of ROS/lipid peroxidation or genotoxicity at lower systemic Co doses in humans.

Proposed mechanisms of action for effects observed in historical cobalt-exposed cohorts: risk factors and susceptibility

The dose–response relationship for Co-related effects does not appear to be the same for all humans. The infrequent occurrence of serious health effects associated with Co blood concentrations that do not induce a response in typical persons likely involves relatively rare underlying disease states, and/or a unique confluence of events involving perturbed Co(II) kinetics that leads to Co toxicity. Below we describe how the plausible mechanisms of action and risk factors may explain individual susceptibility to the adverse actions of Co.

The cobalt beer drinkers

Irreversible and lethal cardiomyopathy, the most severe toxic effect of Co in humans, was observed in a subset of Co beer drinkers. While the actual Co concentrations in the beer and the individual Co doses are not known with a high degree of accuracy, there are some direct measures of Co exposure in this group. For example, in Sullivan et al. (1968), a mean value of 0.48 ± 0.24 μg Co/g wet weight was reported in the cardiac tissue of the Co beer drinkers, 12-fold higher than the mean of 0.04 ± 0.04 μg Co/g in the control cardiac tissue. However, it is important to note that a 12-fold increase in cardiac Co concentrations is probably not sufficient to cause a lethal destruction of heart tissue. Notably, Clyne et al. (1990) reported no mitochondrial inhibition at a 70-fold increase (versus untreated controls) in myocardial Co concentrations in rats treated with Co. Several overlapping factors, including lowered albumin production, chronic ischemic damage and/or oxidative stress, and cumulative organ damage from chronic, severe alcoholism may best explain the increased susceptibility of the Co beer drinkers. As illustrated in Figure 10, a much greater increase in hemoglobin levels per daily Co dose is seen in the Co beer drinker cohort as compared to healthy individuals (and other selected groups).

This working hypothesis is supported by several observations. With respect to hypoalbuminemia, the severely affected individuals were generally alcoholics with liver disease, a group known to exhibit protein malnutrition, which can lead to chronic anemia and hypoalbuminemia (Das & Vasudevan, 2005; Krantz et al., 2005; Niemela, 2007). The Co beer drinkers were reported to be anorexic, and their diets were particularly lacking in protein and thiamine intake (Kesteloot et al., 1968; Alexander, 1972). Many of these individuals also suffered from severe hepatic necrosis and cirrhosis likely due to alcoholic liver disease. Liver disease has been shown to result in impaired ACB (Chen et al., 2011; Jalan et al., 2009). As discussed earlier, a vast majority of the Co in the bloodstream and tissues of normal individuals is bound to albumin, but individuals with hypoalbuminemia would likely experience significant increases in free Co ion levels if the decrease in albumin was sufficiently large (although the magnitude of albumin decrease required to cause a clinically important increase in free Co(II) ions is not yet known).

It is also likely that some fraction of the serum albumin in these individuals was of the damaged IMA variety that reduces the serum-binding capacity for Co. Specifically, alcoholism and poor nutrition alone can lead to cardiac disease as a result of sustained oxidative stress and frequent anemia, which would likely have increased the IMA levels in this cohort. Chronic alcoholics also frequently exhibit acidosis, which often shifts the blood equilibrium towards greater free Co(II) concentrations due to higher levels of IMA as a result of the acidosis. Figure 11 shows that patients with hepatitis, cirrhosis and uremia have significantly higher IMA/albumin ratios when compared to healthy individuals (Chen et al., 2011). In summary, severe liver disease augmented by hypoalbuminemia and increased IMA levels together may have rendered the affected subset of Co beer drinkers more susceptible to Co toxicity due to elevated levels of free Co(II) ion.

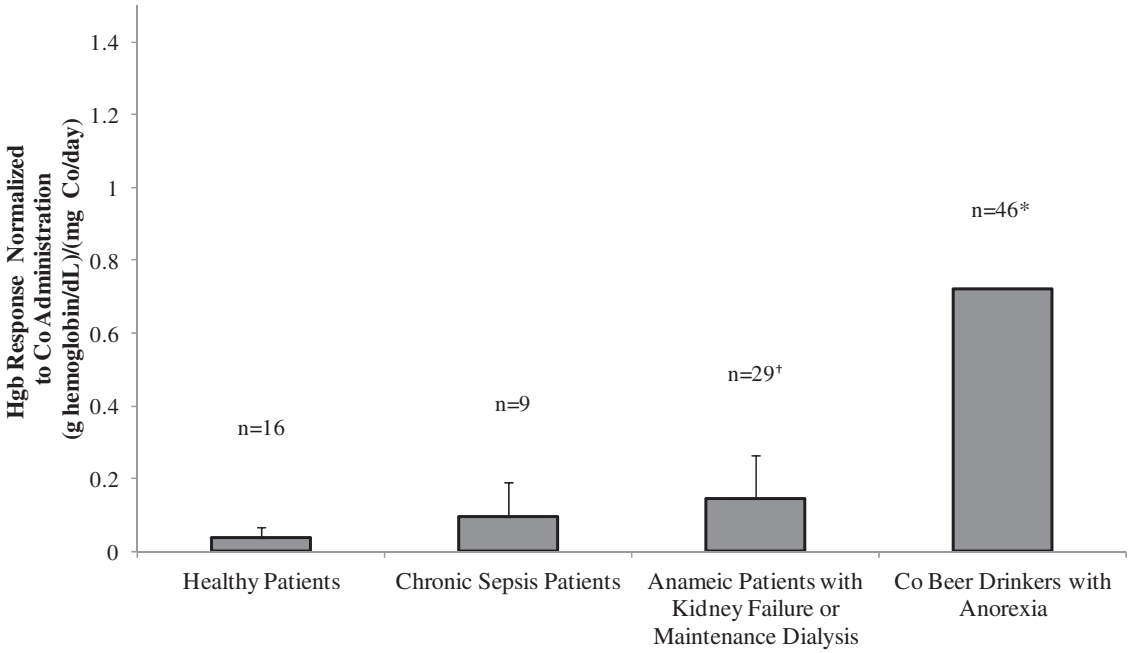

Figure 10. Changes in hemoglobin levels in adults following cobalt exposure. The response is normalized to the Co dose/d. Chronic alcoholics that suffered from anorexia showed a greater response, as indicated by a greater change in Hgb level, than healthy adults treated with Co. Healthy patient data was obtained from Berk et al. (1949); sepsis patient data were obtained from Robinson et al. (1949); anemic patients on maintenance hemodialysis was obtained from Schrirmacher (1967); Duckham & Lee (1976); Schleisner (1956). Beer drinker data was obtained from Kesteloot et al. (1968) and Alexander (1972).
*Original data not available to calculate standard deviation. Initial Hgb levels estimated from data for chronic alcohol consumers. †23 patients for a total of 29 treatment schedules.

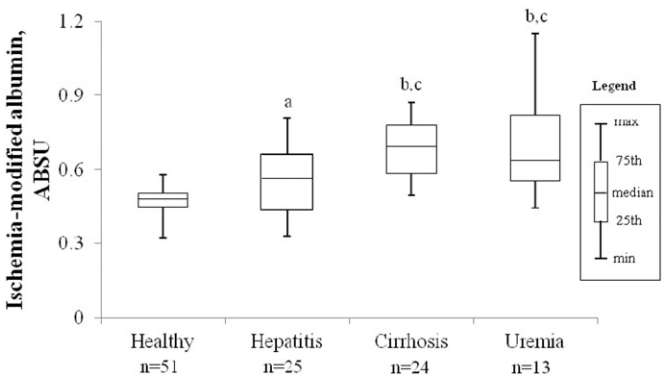

Figure 11. Serum ischemia-modified albumin (IMA) box and whisker plots for healthy controls and patients characterized by various disease states. a and b indicate $p<0.01$ and 0.001, respectively vs. healthy controls; c indicates $p<0.001$ vs. chronic hepatitis patients by one-way ANOVA and LSD test; absorbance unit. Figure adapted from Chen et al. (2011).

Regarding the ultimate mechanism of toxic action of the elevated levels of free Co(II) ion, pathology findings suggest that disrupted mitochondrial function played a pivotal role in the etiology of the cardiomyopathy. In a study of 28 cases of Co beer drinker's cardiomyopathy, electron microscopy of myocardial tissue from these patients showed extensive myofibril degeneration with abnormal mitochondria containing electron-dense bodies believed to contain Co (Alexander, 1972). The researcher suggested that, in the case of beer drinkers' cardiomyopathy, Co depressed mitochondrial oxygen uptake in the myocardium by complexing with sulfhydryl groups (e.g., lipoic acid) and preventing the oxidation of pyruvate in the citric acid cycle (Alexander, 1972). Experimental and clinical studies have reported the protective action of proteins and amino acids, especially if they were rich in SH and NH_2 groups, suggesting that sulfhydryl-rich proteins act as sequestering agents for free Co(II) ions (Seghizzi et al., 1994; Wiberg et al., 1962, 1969). Another suggested molecular mechanism of action for Co beer-drinker's cardiomyopathy includes disruption of intracellular Ca^{2+} concentrations within the heart (Barceloux, 1999; Ramos et al., 2001; Seghizzi et al., 1994).

Other lines of evidence indicate that factors other than elevated free Co were responsible for the cardiomyopathic effects. As shown in Table 7, the estimated blood Co concentrations in the beer drinkers were only 15–180 μg/L. However, it is unlikely that these levels were high enough to have been the sole cause of cardiac tissue destruction, even assuming that all of the Co was in the free ion form. For example, historical Co therapy for anemia typically resulted in blood Co concentrations of 300–900 μg/L (Figure 2) and, since it is known that approximately 5%–10% is in the free ion form (up to blood Co concentrations of 3000 μg/L) based on titration curves and recent measurements, it can be inferred that the free Co(II) ion concentrations in the anemia cohorts were approximately 15–90 μg Co/L. Yet, cardiac changes were rarely reported in anemia patients receiving Co therapy. Further, Kesteloot et al. (1968) studied two groups of Co beer-drinkers; the group that developed cardiac dysfunction had a grossly inadequate diet, particularly lacking in proteins, while the group that was well-nourished did not develop cardiac problems (Kesteloot et al., 1968). These results suggest that

the cardiomyopathic effect may have been partially mediated by a shift in the free versus bound ratio, which resulted in more free Co ions than what would be expected in a well-nourished healthy individual.

Cobalt treatment in renal failure patients

Another example of the likely rare confluence of hypoalbuminemia, greatly increased IMA, and elevated blood Co is suggested by a few cases of neurotoxicity and cardiotoxicity reported among end-stage renal disease patients treated with $CoCl_2$ to resolve anemia. Unlike the beer drinker cohorts, the blood Co concentrations in these patients were very high (ranging up to 2100 μg/L) due to the poor renal clearance of the systemic Co. Diseased kidneys can produce ischemia, as well as, both acute and chronic inflammation (Mawanda et al., 2011) that may increase IMA as reflected in the results of the ACB test (Cichota et al., 2008; Kiyici et al., 2010). Renal failure is also accompanied by increased levels of oxidative stress and impaired efficiency of antioxidant defenses. The need for a permanent fistula or catheter for conducting regular dialysis carries higher risks for local and systemic blood infections that can acutely increase IMA, and partially functioning kidneys in chronic renal disease patients can exhibit proteinuria that includes albumin loss and decreasing total albumin levels (Cichota et al., 2008). Finally, chronic renal failure leads to the need for dialysis, a process that adversely affects albumin in several ways. Dialysis patients are directed to eat lower protein diets that may lead to varied degrees of protein malnutrition. These patients often overhydrate, which dilutes the existing albumin in the total blood volume and affects ion equilibrium. Dialysis inherently leads to albumin loss from repeated filtration of the blood, in addition to blood cell stress that leads to increased hemolysis and greater extracellular Fe and hemoglobin in the blood that may affect divalent cation balance. Taken together, not only are anephric patients likely to have higher Co blood concentrations than those seen in healthy individuals, they are also likely to have a higher free Co(II)/bound Co serum ratio.

Cobalt treatment in severe sepsis patients

Several risk factors can render patients with acute or chronic sepsis more susceptible to the adverse effects of Co (Robinson et al., 1949). First, the presence of chronic inflammation and acute flares of inflammation related to infection leads to oxidative stress, which subsequently can increase IMA levels. Second, in some individuals with these sepsis conditions, the gastrointestinal tract is the source of continuing infection, and there may be surgical or physiological alterations leading to protein malnutrition, which may cause hypoalbuminemia. Third, these patients are commonly under chronic treatment with antibiotic medications that may further augment oxidative stress and IMA accumulation. Each of these factors acting in the presence of a high internal dose of Co may result in an equilibrium shift towards greater free Co(II) ions in blood and tissues. Interestingly, while this can lead to adverse effects at high doses, it can also result in increased concentrations of hemoglobin at lower doses of Co than those required to elicit a similar effect in healthy individuals. As illustrated in Figure 10, a greater therapeutic response of sepsis patients to the hemoglobin stimulation effects of Co is observed relative to other selected groups (Berk et al., 1949; Robinson et al., 1949). It is notable that while therapeutic effects were observed at lower doses than normal in this cohort, no adverse effects were reported in these patients (Robinson et al., 1949).

Cobalt treatment in sickle cell anemia patients

A dose–response trend for diminishing thyroidal iodine uptake with increasing Co dose in children treated with Co is illustrated in Figure 12. This response was far more severe in sickle cell children treated with similar doses (1–2.5 mg Co/kg-d), and led to goiter development and decreased iodine uptake. A plausible explanation for such increased susceptibility can be surmised from the natural history of this disease and its potential impact on free Co(II) equilibrium in the blood. Specifically, sickle cell disease involves increased blood cell stress, similar to the effects of chronic dialysis, leading to increased hemolysis and greater extracellular Fe and hemoglobin in blood, which can affect the divalent cation balance (Chan et al., 1999). Sickle cell disease also often involves glomerular membrane damage due to renal hyperperfusion and hyperfiltration, which may involve proteinuria and hypoalbuminemia, as well as increased IMA from renal inflammatory responses. Further, sickle cell crisis may involve an acute flare of inflammatory responses affecting numerous tissues in the body, and likely leading to oxidative stress and IMA (Nur et al., 2011). Markers of oxidative stress have been shown to be elevated in sickle cell patients as compared to healthy individuals, and increased levels of ROS leads to increased levels of IMA and potentially greater free Co(II) concentrations (Nur et al., 2011).

Cobalt treatment and severe protein malnutrition or hypoalbuminemia

Patients who suffer from severe protein malnutrition may develop hypoalbuminemia due to a severe deficiency in proteins. As a result, albumin synthesis is decreased (Krantz et al., 2005). For example, the Co beer drinkers were reported to be severely malnourished; their diets were particularly lacking in protein and thiamine intake (Kesteloot et al., 1968; Alexander, 1972). Kesteloot et al. (1968) studied two groups of Co beer-drinkers; the group that developed cardiac dysfunctions had a grossly inadequate diet, particularly lacking in proteins, while the group that was well-nourished did not develop cardiac problems (Kesteloot et al., 1968). Further, two children described by Tevetoglu et al. (1956) who showed a very high response to Co exposure as indicated by changes in hemoglobin levels were described as being severely malnourished. Yet there were no cardiac complications noted in these malnourished children with higher responses to lower doses of Co. Thus, poor diets, particularly lacking in protein, may lead to decreased total albumin levels, which may result in increased free Co(II) ions. For example, the dose–response trend for increasing hemoglobin levels relative to Co dose is illustrated in Figure 13.

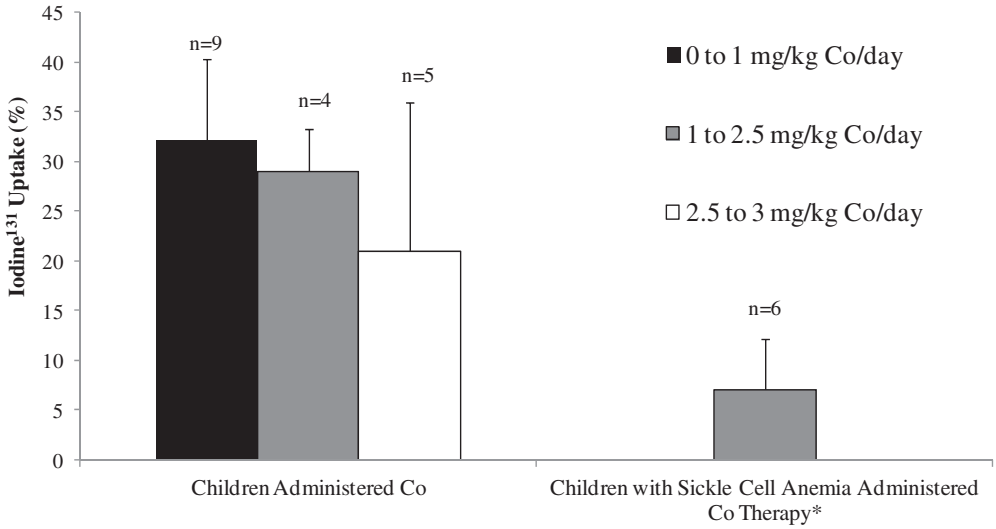

Figure 12. Iodine uptake in children following Co exposure. Iodine uptake was greatly reduced in sickle cell children relative to healthy children that received similar doses. Exposure lengths varied. Healthy children data were obtained from Jaimet & Thode (1955) following 5 weeks of dosing in children ranging in age from 5 to 9 years. Sickle cell data came from Kriss et al. (1955); Gross et al. (1955) and Keitel (1955). Ages ranged from 3.5 to 19-year-old and duration of exposure ranged from approximately 5 weeks to 7 months. The iodine uptake in children reported by Jaimet & Thode (1955) did not differ greatly between 5 weeks and 10 weeks of exposure for the two lowest dose categories. For example, the average iodine uptake after 5 weeks of exposure to 0.45 to 1 mg/kg-d was 32% as compared to 31% at 10 weeks. After 5 weeks of exposure to 1 to 2.5 mg/kg-d the average iodine uptake was 29% as compared to approximately 27% after 10 weeks.
*Four patients for a total of 6 treatment schedules.

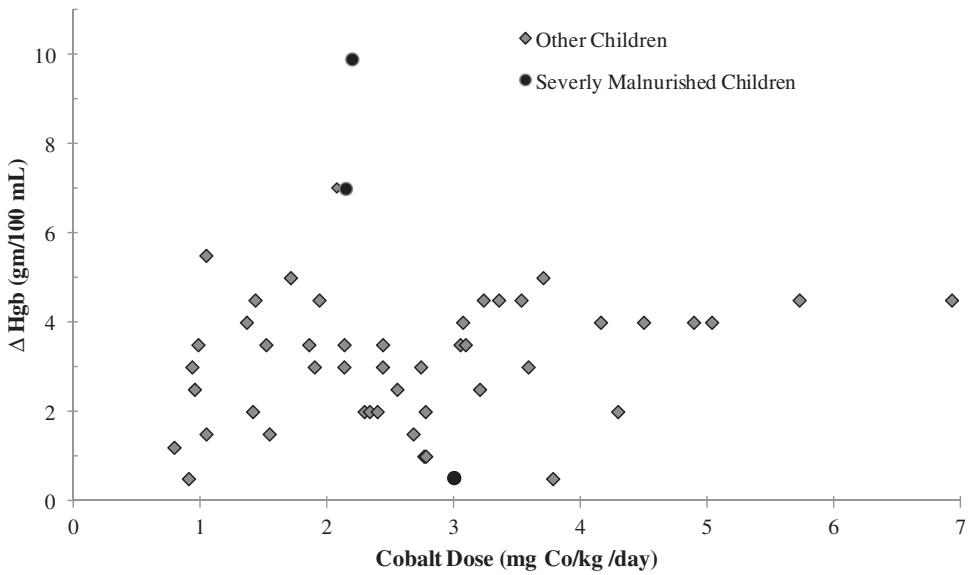

Figure 13. Changes in hemoglobin levels in infants and children following cobalt exposure. Two of the three children described as being severely malnourished showed a greater change in Hgb levels following Co therapy relative to other children studied. The other high responder was identified as one of 24 children with anemia associated with infection. Children data were obtained from Tevetoglu et al. (1956) following 4–14 weeks of dosing in children ranging in age from 1 month–10 years. Two of the three high-responders were described as severely malnourished.

Summary

Based on our evaluation of the literature, it seems clear that free Co(II) is an important factor in determining the magnitude of response for a given Co dose. Individuals who suffer from decreased albumin levels or increased concentrations of IMA may experience higher levels of free Co in target organ tissues, and thus elicit a greater response at lower doses. It is therefore likely that anephric patients, sepsis patients, sickle cell children and Co-beer drinkers had higher levels of free Co(II) due to increased levels of IMA and/or lower levels of albumin, resulting in toxicity at doses (e.g., blood concentrations) that have not been observed to cause adverse effects in otherwise healthy persons. These populations apparently demonstrate a left-shifted dose–response relationship for the most sensitive endpoints indicating Co(II) ion response (hypothyroidism and polycythemia). However, only the combination of chronic alcoholism, severe malnutrition

and elevated Co exposure in the beer drinkers' cohort has been clearly associated with the deadly cardiomyopathy response, suggesting that chronic alcoholism likely played an important role in its etiology.

Evaluation of current cobalt-exposed populations

As discussed earlier, the use of Co as a therapeutic agent to treat anemia has been discontinued. Some reviewers suggest this occurred due to inconsistent efficacy and reports of adverse thyroid effects in children (Carson et al., 1986; Fisher, 1998), while others suggest Co therapy was simply replaced by erythropoietin therapy (Cobalt Development Institute (CDI), 2012). It is worth noting that as recently as 1978, it was suggested that a ''single oral dose of 150 mg $CoCl_2$ in man is harmless except for polycythemia'' (Venugopal & Luckey, 1978).

Currently, certain subpopulations continue to experience Co exposures that are well above those associated with normal dietary intake: individuals taking Co supplements, women on Co therapy to control estrogen excretion and patients with Co-containing hip implants. In the following section, for each of these groups we review: (1) the range of measured and/or estimated blood Co concentrations; (2) whether any Co-related adverse health effects have been reported or would be expected to occur in the population; and (3) the degree to which Co may have contributed to any such effects. We also discuss whether potentially susceptible individuals might be expected to exist in these populations as a result of reduced serum protein binding or other factors.

Dietary cobalt supplements

A number of Co-containing dietary supplements are available for sale in the US, with recommended daily doses ranging from 200 to 1000 μg of Co/d (DRN, 2012; MEMI, 2011; Mineralife, 2012). A 2002 health and diet survey conducted by the FDA indicated that 0.07% of 888 people surveyed had taken a Co dietary supplement in the past 12 months (Lin, 2007). In addition, some energy drink products contain vitamin B_{12} in amounts as high as 41 677% of the US FDA Daily Value of 6 μg (Zipfizz Corp, 2011), which corresponds to an intake of about 100 μg Co per serving.

These doses are all lower than the Co dose (1400 μg Co/d in a 60 kg adult) identified by the UK Expert Group on Vitamins and Minerals to be unlikely to cause adverse health effects in humans. Some of these Co supplement doses are also lower than the safety value of 600 μg Co/d suggested by the European Food Safety Authority (EFSA) for non-cancer effects. Further, these doses are lower than the Co reference dose of 2100 μg/d (for a 70 kg adult) recommended by Finley et al. (2012b). In short, at doses normally associated with dietary supplementation ($\leq$1 mg Co/d) adverse health effects would not be expected based on the available literature.

This view is supported by recent human volunteer studies in which blood Co concentrations were measured in individuals consuming Co supplements. For example, Tvermoes et al. (2013b) reported that consumption of 400 μg Co/d by four healthy male adults for approximately 2 weeks resulted in peak whole blood Co concentrations ranging from 1.8 to 5.1 μg Co/L (Figure 7) and no adverse health effects were reported by the volunteers (all four males were ChemRisk employees). Preliminary data are available from two additional Co supplement studies in human volunteers ingesting $\sim$1 mg Co/d with dosing durations of approximately 30 d and 90 d. The 30 d study included five male and five female volunteers with peak whole blood Co concentrations ranging from 9.6 to 34.6 μg/L for males and from 6.3 to 91.4 μg/L for females (Figure 9) (Tvermoes et al., 2013a). The 90-d study is still in progress but peak whole blood Co concentrations ranging from 12.4 to 37.7 μg/L and 9.4 to 117.2 μg/L has been reported for five males and four females, respectively (Paustenbach et al., 2013). Detailed findings of the 30- and 90-d Co supplement studies will be reported separately.

It is possible that some individuals may consume Co supplements at doses far beyond those recommended on the label. For example, Simonsen et al. (2012) noted that suspicion has been raised about the possible misuse of Co by endurance athletes as part of their conditioning to optimize tissue oxygenation during long and strenuous exercise events. There are some acceptable upper limits for professional athletes regarding RBC and hematocrit levels. Although different governing bodies for endurance sports have different limits and monitoring guidelines, traditionally male and female hematocrit levels could not exceed 50% and 47%, respectively. For RBCs, the number of mature and immature RBCs in circulation are commonly examined (in a normal person, the fraction of immature cells divided by mature cells is less than 1.0). While it is clearly possible to ingest therapeutic (polycythemic) doses of Co without experiencing serious adverse effects, and although there is no reason to suspect that endurance athletes might exhibit any of the afore mentioned risk factors, this might be an area that warrants further investigation.

Cobalt therapy

Although not common, daily Co doses ranging from 500 to 1120 μg Co/d have been recommended by some homeopathic doctors to correct excessive excretion of estrogen that sometimes occurs during female hormone replacement therapy (Wright, 2005). These Co doses are similar to those taken by individuals seeking health benefits from off-the-shelf supplements and, therefore, we would not expect this form of therapy to pose a risk to an otherwise healthy adult female. In addition, current research is investigating the use of Co-complexes as potential pharmaceutical agents for the treatment of HIV, as an alternative to platinum-based chemotherapeutics, and to improve the therapeutic efficacy of small molecule drugs such as non-steroidal anti-inflammatory drugs (Heffern et al., 2012; Ott & Gust, 2007). Further, a Co(III)-based pharmaceutical to treat drug-resistant herpes simplex virus 1 has made it to clinical trials (Heffern et al., 2012). Thus, the therapeutic benefits of Co continue to be explored today.

Cobalt-containing hip implants

Co-containing alloys have been utilized for decades in orthopedic prosthetics such as knee and hip implants (Marti, 2000), and it has long been understood that metal-containing

implants release metals into the bloodstream through a combination of corrosion and wear (Jacobs et al., 1998).

MoM hip implant patients have blood Co concentrations typically ranging between 0.1 and 10 μg/L (Antoniou et al., 2008; Brodner et al., 2003; Engh Jr et al., 2009; MacDonald et al., 2003; Vendittoli et al., 2007; Walter et al., 2008). These concentrations are less than the mean values measured in males and females (16 μg Co/L and 33 μg Co/L, respectively) after ingesting approximately 1.0 mg Co/d of a commercially available Co supplement for an average of 31 d.

There are several recent case reports of adverse effects occurring in implant patients with blood Co concentrations ranging from 14 to 6521 μg/L (Table 10). In our search for historical case reports, we identified 17 individual cases (some cases were reported more than once in the literature) that contained reasonably detailed information to offer insights on potential systemic health effects. Nearly all the case reports (15 of 17) occurred in the recent literature since 2006. The distribution of bearing surfaces involved in the studies was: eight MoM, six metal-on-polyethylene (MoP), two metal on ceramic (MoC) and one unspecified type. The age distribution included seven cases aged 55 or less, six cases aged 56–65 and four cases aged above 65. Most cases were male (10 of 17) with an average age of 58.1, while the seven female cases had an average age of 60. Exceptionally high blood or serum Co concentrations were associated in some cases with concurrent or prior failed ceramic bearings that led to a rapid deterioration of the metal bearing surface due to the presence of ceramic debris. For example, Zywiel et al. (2013) reported a fatal case that included anorexia, hypothyroidism and cardiomyopathy in an individual with a MoP bearing that reportedly lost >28 g of metal mass due to ceramic particle wear and resulted in a peak blood Co concentration of 6521 μg/L. Another failed MoC bearing was reported to have lost 79 g of metal mass associated with a peak blood Co concentration of 398 μg/L (Steens et al., 2006). With the exception of the fatal case that occurred in a 52-year-old male with a peak Co blood concentration of 6521 μg/L, the adverse effects in most other cases were reported to be partially or fully reversible after implant revision (Table 10).

When examining Table 10, it is notable that four of the case reports did not provide any measurements of blood or serum Co concentrations, and about half of the cases (7 of 13 with reported Co concentrations) were about 400 μg/L or higher. These findings appear to be consistent with other reports of primarily reversible thyroid and neurological effects occurring among anephric individuals receiving Co therapy for anemia who likely exhibited comparable blood Co concentrations as discussed earlier (Bowie & Hurley, 1975; Duckham & Lee, 1976).

It is important to consider that some of these case reports provide very little detail regarding the exact nature and course of the reported clinical consequences (e.g., Tower et al. (2010a,b)) and underlying disease states and other factors that are now known to be related to significant metal bearing wear. For example, Ng et al. (2013) reported 3 weeks of blurred vision in the left eye of a 39-year-old female with relatively low Co serum concentrations (~45 μg/L); no clinical progression was noted at a 1 month and 6 month follow-up, and the unilateral occurrence seems inconsistent with a systemic Co effect. Neurological symptoms were also reported in a 73-year-old female with low Co serum concentrations (~24 μg/L); however, the symptoms were reported to occur after a cerebrovascular event (consistent with a stroke) which may have contributed to her cognitive decline and memory loss.

No detailed discussion of possible pharmaceutical agents being taken by these patients was provided; which may account for some of the related symptoms. Four of the cases in Table 10 reportedly occurred in individuals with diabetes mellitus (Janicek et al., 2012; Katzner & Schvingt, 1983; Machado et al., 2012; Zywiel et al., 2013), which, in more advanced and uncontrolled stages, may increase IMA and possibly induce perturbations in Co(II) kinetics as explained earlier. However, the types and severity of diseases that can significantly affect free Co(II) concentrations and susceptibility have not been clearly defined to date. It is hoped that new analytical tools like the Co speciation assay of Kerger et al. (2013a) can provide insights on these conditions by discerning those persons with exceptionally low ACB capacity in serum. Specifically, a more detailed understanding of the factors leading to the perturbation of Co kinetics might help to discern whether the lower blood Co concentrations identified in some of the cases in Table 10 are explained by Co or perhaps due to other or unknown factors.

Cancer endpoints. Markers of genotoxicity have been investigated in some MoM hip implant patients, but the clinical significance of these findings is not known. For example, a study by Stea et al. (2000) reported that sister chromatid exchange levels in peripheral lymphocytes of patients with CoCr-containing implants were higher but not significantly different from controls. Additionally, Ladon et al. (2004) reported no correlation between blood Co concentrations and chromosomal translocation in patients with Co-containing implants. Davies et al. (2005) reported an increase in DNA damage in human fibroblasts in tissue culture after an exposure to synovial fluids isolated from patients containing MoM hips. Similarly, Daley et al. (2004) reported that isolated-wear debris from patients with CoCr metal hip or knee alloys induce chromosomal damage in tissue culture. However, these results do not imply that MoM hip implant patients are at a greater risk for malignancies because tissue-culture systems are not a precise model or predictor of what happens *in vivo*. Further, molecular epidemiological studies suggest that DNA damage and chromosomal aberrations are imprecise indicators of an increased cancer risk (Collins et al., 1997).

While it has been noted that certain forms of Co are classified by the International Agency for Research on Cancer as ''possible,'' ''probable,'' or ''known'' human carcinogens (IARC, 2006; Polyzois et al., 2012), the evidence reviewed by IARC (2006) pertains primarily to chronic inhalation of fine metal powders (hard metals with tungsten carbide and Co) or Co pigments at relatively high doses that accumulate in the lungs and induce ''portal-of-entry'' carcinogenesis in the form of lung cancer. Lung-specific cancer is not unusual, at some dose, for substances that are inhaled that are genotoxic and, frequently, such effects are limited to the lung. In short,

Table 10. Examples of various case reports of adverse effects which may have been associated with various prosthesis types and designs.

Reference	Gender/Age	Type of prosthesis	Duration of exposure (Months)	Reported peak Co blood or serum concentration (μg/L)	Reported health effects
Tower et al. (2010b)	M/49	MoM	43	122‡	Rash, dyspnea, tinnitus, high-frequency hearing loss, pain, hand tremor, cognitive decline, depression, optic nerve atrophy, diastolic dysfunction and metallosis
Tower et al. (2010b)	M/49	MoM	40	23‡	Cognitive decline, vertigo, hearing loss, dyspnea, groin pain, rashes, psuedotumor, metallosis and pseudocapsule
Steens et al. (2006)	M/53	MoC	24	398*,‡	Hearing and vision impairment, numbness in his feet, dermatitis, optic atrophy as well as localized soft tissue reaction
Oldenburg et al. (2009)	M/55	MoP	3	625†	Hypothyroidism, peripheral neuropathy, cardiomyopathy, eczema, progressive hearing loss, fatigue, poor concentration and metallosis
Ikeda et al. (2010)	F/56	MoP	24	>400†	Sensory disturbance, neuropathy, numbness, tingling, hearing loss and hypothyroidism
Harvie et al. (2008)	F/64	MoM	24	NR	Impaired femoral nerve function with evidence of complete nerve destruction due to encasement within the pseudotumor
Harvie et al. (2008)	F/55	MoM	36	NR	Femoral nerve damage due to encasement within the pseudotumor
Catalani et al. (2011); Rizzetti et al. (2009) and Pazzaglia et al. (2011)	F/58	MoP	5	549†	Hypothyroidism, blindness, deaf, bilateral cranial nerve impairment, mild distal sensory-motor disturbances, as well as optic, acoustic and peripheral neuropathy
Matziolis et al. (2003)	F/75	MoP	9	NR	Pain and metallosis of the pericapsular soft tissues
Janicek et al. (2012); Pelclova et al. (2012)	M/56	MoP	20	506‡	Weight loss, quadriparesis, hypothyreosis, cardiomyopathy, exanthema and perceptive amblyacousia
Mao et al. (2011)	F/73	MoM	60	24‡	Neurological symptoms, cognitive decline, memory difficulties, depression, metallic taste, headaches, anorexia and mild groin pain
Mao et al. (2011)	M/60	MoM	48	15‡	Muscle fatigue with pain, cramps in his hands and feet, dysponea, problem remembering names and uncontrolled hypertension
Katzner & Schvignt (1983)	M/67	not specified	22	NR	Acetabular lytic lesion and pain, biopsy of the lesion shows a metastatic lesion supposed of renal origin
Apel et al. (2012)	M/65	MoC	72	446.6‡	Vision decline, general malaise, pericardiomyopathy, paroxysmal atrial fibrillation, bulbar palsy, pulmonary embolism and motor axonopathy
Zywiel et al. (2013); Gilbert et al. (2013)	M/52	MoP	14	6521†	Fatigue, tinnitus, painful mass proximal to left hip, anorexia, hypothyroidism, cardiomyopathy and death
Machado et al. (2012)	M/75	MoM	96	14‡	Cardiomyopathy
Ng et al. (2013)	F/39	MoM	60	45‡	Blurred vision, metallic taste in mouth, nausea

*Concentration reported shortly after revision.
†Reported Co whole blood concentration.
‡Reported Co serum/plasma concentrations.
NR: Co blood or serum concentration was not reported.

there has been no indication of an increase in site-specific cancers outside of the respiratory tract which has been observed for these occupational groups exposed to Co pigments and metal dusts (IARC, 2006). As such, the findings on the increased lung cancer risk related to the inhalation of Co(II) does not suggest a systemic cancer risk due to MoM or MoP implants.

Substantial research has been conducted to evaluate the prevalence of numerous cancers among populations with MoM hip implants, including those with Co-containing prosthetics. To date, the results do not appear to indicate an increased risk of cancer. Hip implant cohorts in Denmark (Olsen et al., 1999; Visuri et al., 2003, 2006a), England and Wales (Smith et al., 2012), Finland (Paavolainen et al., 1999a,b; Visuri & Koskenvuo, 1991; Visuri et al., 1996, 2003, 2006a, 2010a,b), New Zealand (Gillespie et al., 1988) and Sweden (Mathiesen et al., 1995; Nyren et al., 1995; Signorello et al., 2001; Visuri et al., 2006b) have been evaluated, and the weight of evidence indicates that metal-containing hip implants do not pose a cancer risk to patients. In the meta-analysis conducted by Visuri et al. (2006b), the authors concluded that for patients who underwent total hip replacements the ''[c]ancer incidence was in line with the general population'' (2006b). In many cases, cancer rates were actually significantly lower in the implant population relative to the general population. Follow-up studies with longer latency periods would be useful.

Although many of the epidemiologic assessments of hip implant cohorts did not present findings specific to patients with MoM bearings, the assessments that did stratify by implant type consistently showed a lack of an association between risk of cancer and having a MoM implant (Smith et al., 2012; Visuri et al., 1996, 2010b). For example, a recent study conducted by Smith et al. (2012) compared second-generation MoM bearings to non-MoM bearings and concluded that ''there was no evidence that metal-on-metal bearing surfaces were associated with increased risk of any cancer'' (Smith et al., 2012). Further, relative to the Smith et al. (2012) assessment, Visuri et al. (2010b) included a longer follow-up time in their evaluation of cancer risk among a Finnish-based cohort with MoM bearings. Similar to Smith et al. (2012), Visuri et al. (2010b) concluded that MoM or MoP ''prostheses do not expose patients to an increased risk of cancer'' (Visuri et al., 2010b, p. 6). Contrary to the Smith cohort, Visuri et al. (2010b), examined patients with first-generation MoM bearings, which they noted had worse wear properties than second-generation bearings. As such, Visuri et al. stated that ''if excessive metal ion loading from the [first-generation] components did not increase risk of mortality within this cohort, such risk would not be obvious in recipients of [MoM] implants with improved wear characteristics'' (Visuri et al., 2010b, p. 4).

Overall, the epidemiologic research conducted over various geographic, temporal and demographic settings has failed to demonstrate an increased risk of cancer among hip prosthesis patients. Unfortunately, the percentage of the study populations with Co–Cr prostheses, and the blood Co concentrations in these patients were not reported in these studies. However, virtually all metal-containing hip prostheses contain Co–Cr components that can result in elevated blood Co concentrations. Accordingly, the broader population studies on hip prosthesis patients (including MoM and other bearing types) are relevant to the question of potential carcinogenic risks of chronic, systemic Co exposures.

Pseudotumors. Some investigators have suggested that local cystic masses that form in some MoM prosthesis patients (often referred to as ''pseudotumors'') may be a sensitization and/or inflammation response to the accumulation of Co in the hip synovial fluid. Co concentrations in hip synovial fluid of failing hip prostheses have been reported to range from 589 to 3300 μg Co/L (Kwon et al., 2011; Davda et al., 2011; Tower, 2010a,b). However, pseudotumors also form in patients with non-MoM implants and in MoM patients with no unusual wear (Campbell et al., 2010; Howie et al., 1991; Leigh et al., 2008; Malviya et al., 2009). Thus, it remains unclear whether these localized responses are triggered by wear- or corrosion-related accumulation of Co (or other metal ions) or some other factor (Natu et al., 2012). Concentrations of Co generally measured in the synovial fluid of well functioning hips are generally below concentrations of Co shown to be cytotoxic *in vitro* (Akabar et al., 2011; Andrews et al., 2011; DeSmet et al., 2008).

Polyzois et al. (2012) suggested that the larger surface area of nanometer range Co-containing wear debris generated from hip implant alloys may be responsible for these local tissue responses (including pseudotumors), and that the responses are a result of cytotoxicity and/or an enhanced immune response to Co-nanoparticles. To date, the weight of evidence remains unclear regarding the role of Co(II) ions and Co nanoparticles in the development of local effects. First, while the smaller size of nanoparticle debris from MoM hip prostheses does create greater surface area of metal debris, this size range is actually less problematic than larger (e.g., micrometer range) wear debris with respect to foreign body clearance mechanisms (Almeida et al., 2011; Natu et al., 2012). Indeed, problematic local tissue responses occur with both MoM and MoP prostheses (the latter generally showing no evidence of excessive metal-wear debris), and have been a relatively infrequent cause (<1%) of implant failure for both types over the past few decades. The local tissue reactions for MoM patients commonly involve prominent histiocytic macrophage and T cell recruitment, as would be expected in response to nanoparticle debris, since histiocytes manage finer foreign bodies while other leukocytes (e.g., neutrophils and giant cells) are selected for managing bacterial infections and larger foreign bodies. Micron-size debris may be more likely to induce the recruitment of neutrophils, macrophages and lymphocytes associated with necrosis and joint pain as seen with crystal-induced arthritides (Reginato, 2005; Rosenberg, 2005).

The relationship of these rare, but clinically important, chronic inflammatory responses at the implant site to Co–Cr nanoparticles or to Co(II) ion release remains unclear because these rare local tissue reactions that are seen in the presence and in the absence of excessive metal-wear debris (Kwon et al., 2010; Pandit et al., 2008a,b; Willert et al., 2005). Moreover, since none of the available *in vivo* or *in vitro* studies have measured Co(II) ion concentrations (only total Co in tissues and body fluids), it remains unclear what role

metal ions play in these responses (as opposed to Co debris, other metals, or debris from other implant-related materials). Some investigators consider these severe chronic inflammatory responses to be associated with rare phenotypes that form secondary lymphoid centers to address chronic antigenicity to prosthesis-related debris, but the excessive fibrotic reactions then cut-off the necessary lymphoid drainage pathways, leading to cystic lesions, fluid build-up and pain (Hayasaka et al., 2010; Natu et al., 2012; Thaunat et al., 2006). Regardless, there is no strong evidence for a role of Co(II) ions at relevant *in vivo* concentrations in the development of these clinically important local tissue responses involving severe tissue necrosis, pain and excessive scar tissue formation around the implant.

Nanoparticle-wear debris from Co–Cr hip prostheses

Recent reviews (Billi & Campbell, 2010; Gill et al., 2012; Keegan et al., 2008; Polyzois et al., 2012) have posited that nanometer-size-wear debris from Co–Cr alloy (MoM) hip prostheses may pose a hazard with respect to both local tissue reactions and systemic toxicity because:

(1) newer generation MoM hip prostheses generate lower total mass and volume of wear debris, but they create far more nanometer-size particles with a far greater particle surface area that may trigger adverse biological interactions with immune cells and other tissues;
(2) the greater fraction of nanometer-size alloy particles can more readily be distributed to other organs that may create secondary sites of toxicity beyond the implant local tissues; and
(3) some *in vitro* studies suggest that nanometer-size particles may be cytotoxic, at some doses, to certain somatic and immune cell lines when compared to micron-size particles.

While these factors, considered alone or in combination, might raise suspicions that Co–Cr alloy nanoparticles could play a role in local tissue reactions, the available research does not support any currently measurable role for such nanoparticles in systemic Co toxicity among hip prosthesis patients.

For example, the two key studies providing evidence of Co–Cr alloy nanoparticle distribution to distant lymph nodes, bone marrow, liver and spleen (Case et al., 1994; Urban et al., 2000) have not provided convincing evidence of associated toxicity of Co or nanoparticles at these distant sites. On the one hand, the smallest nanoparticles, (i.e., those less than about 8 nm in diameter), are known to be cleared by the kidneys (Almeida et al., 2011), while those between 8 nm and about 10 000 nm are primarily managed by the cellular immune system through sequestration, enzymatic dissolution, and/or lymphatic drainage. On the other hand, larger debris particles that are too large to be consumed by macrophage cells (e.g., >10 microns in diameter) can trigger immune responses that are more damaging to local tissues and include fibrotic sequestration reactions including scar tissue formation that can limit prosthetic joint mobility. Importantly, the alloy nanoparticles that occur in higher numbers for newer MoM implants are predominantly within a size range (e.g., 8–100 nm) that can be readily swept up and cleared by cellular immune responses.

While rare situations of rapid wear debris accumulation and/or failed lymphatic drainage might create local tissue immune reactions, there is no convincing data to indicate systemic toxicity from Co–Cr alloy nanoparticles in hip prosthesis patients. Differences in the cellular ''foreign body'' response between individuals may trigger far more severe local tissue responses involving necrosis, fibrosis and cyst formation (Natu et al., 2012), but a specific relationship to Co has not been demonstrated in the large population of patients who have received Co–Cr prostheses. Moreover, enhanced ''foreign body'' responses and associated inflammatory processes have many triggers related to particle size/quantity and antigenicity that occur with other metal alloys, polyethylene, Teflon and other persistent biomaterials (Black et al., 2007; Natu et al., 2012; Reginato, 2005), making it difficult to characterize such responses as representing specific toxicity from the metal ions composing the alloy.

Specific to Co, several studies of wear debris in hip implant patients and in a variety of *in vitro* tests have shown that Co–Cr alloy-wear debris in the nanometer size range is rapidly depleted of Co, leaving largely insoluble Cr(III) oxide shells. Whether this release of Co is related to simple aqueous dissolution and/or extracellular or intracellular corrosion processes, it is clear that Co is only briefly associated with wear debris nanoparticles (Catelas et al., 2004; Doorn et al., 1998; Goode et al., 2012; Pourzal et al., 2011). For example, several *in vitro* studies have examined the dissolution rate of Co from various engineered Co and Cr particles (Germain et al., 2003; Horie et al., 2012; Kwon et al., 2009; Lewis & Heard, 2005; Lewis et al., 2005, 2007) and determined that the vast majority of Co is released within minutes to hours. *In vitro* studies demonstrate that more concentrated exposures to Co(II) ions (e.g., typically >100 μM) can be cytotoxic to immune cells and hepatocytes probably due to mitochondrial toxicity, leading to apoptosis and/or necrosis (Akbar et al., 2011; Battaglia et al., 2009; Huk et al., 2004; Petit et al., 2005); thus, it is plausible that isolated pockets of high Co(II) release may affect local tissues and immune cells where very high concentrations of wear particles accumulate in a short period of time. One must also consider that apoptosis and necrosis are intended responses within the cascade of cellular immune responses, inflammation and healing, and that low level occurrence of these processes *in vivo* may not have important clinical consequences in many situations.

A recent review by Polyzois et al. (2012) discussed the potential risk for retinopathy and hearing difficulties, as well as thyroid and reproductive effects in MoM patients as a result of prosthesis-derived metal debris. However, they acknowledge that the hearing and vision effects were associated with extreme wear of the prosthesis and that no reproductive or developmental effects have been identified in MoM patients (Brodner et al., 2004; Cobb & Schmalzreid, 2006). In fact, two recent papers describe the healthy births of four pregnant patients with MoM hip implants (deSouza et al., 2012; Fritzsche et al., 2012). It is important to keep in mind that the *in vivo* hazard of nanoparticle kinetics and corrosion is complex and dynamic, and that excessive tissue Co accumulation (e.g., >100 μM or 5900 μg Co/L) in organs distant from the implant site has not been reported and seems unlikely. Once released from nanoparticles, the Co(II) ions bind with

proteins in synovial fluid and adjacent tissue surfaces (Lewis & Heard, 2005; Lewis et al., 2005) and ultimately distribute into peripheral blood, which has a high capacity for Co–protein binding and sequestration. The potential for Co cytotoxicity, either in local tissues, immune cells, or in more distant tissues, depends on the complex kinetics that begin with identifying the rate of wear debris generation and the associated rate of Co(II) ion release from that debris. Even if those two rates were well characterized (which they are not), the subsequent protein-binding interactions of Co(II) ions and the rate of distribution into peripheral blood and tissues makes for an inherently complex kinetic modeling exercise. The most likely common denominator to systemic Co toxicity in patients with high-wear debris is understanding the Co–albumin-binding capacity at plausible rates of distribution of Co between local tissues and the blood.

Based on the results of *in vitro* studies, Polyzois et al. (2012) suspect that Co nanoparticles released from hip implants are likely to cause cytotoxicity or apoptosis in endothelial cells and lymphoid progenitor cells. However, the potential role of nanoparticles and/or associated release of Co(II) ions in Co–Cr hip prosthesis patients is not likely to be addressed by available *in vitro* studies because these systems do not adequately simulate the dynamic conditions known to occur *in vivo*. For example Kwon et al. (2009) reported a significant dose-dependent reduction in macrophage viability with increasing concentration of Co nanoparticles (starting at 1 trillion particles per mL, 30–60 nm diameter) and Co(II) ions (starting at 1 mM or 59 000 μg Co/L), but effects of these extremely high concentrations in static systems are not highly relevant to the dynamic *in vivo* situations for hip prosthesis patients. Similarly, Papageorgiou et al. (2007) reported that Co(II) ions were released more rapidly from smaller nanoparticles compared to micron-size particles, but again the rates of debris generation, Co dissolution, and multiple binding and clearance mechanisms in dynamic equilibrium *in vivo* make it difficult to assign great relevance to these *in vitro* findings.

Goode et al. (2012) analyzed the hip capsule tissue from a MoM hip implant patient undergoing revision surgery and these two types of wear debris were present: a diffuse phase containing mainly Cr^{3+}, no Cr^{6+}, and only trace amounts of oxidized Co, as well as metallic particles containing Co, Cr and Mo. The authors noted that the particles found inside the macrophages contained little Co, suggesting that Co^{2+} was released from the metallic particles prior to macrophage ingestion (Goode et al., 2012). Nanoparticles are likely to undergo other complex interactions *in vivo*, such as agglomeration and protein absorption that cannot be readily characterized with *in vitro* systems. Thus, whether or not nanoparticles may play some role with respect to the possible systemic hazards of Co released from MoM hip prosthesis will require additional research.

Conclusions, recommendations and areas for future research

Several recent studies involving Co exposure metrics have yielded a more comprehensive understanding of Co dose–response relationships for various adverse effects. In particular, the biokinetic model of Unice et al. (2012) permits estimation of blood Co concentrations following oral exposures to Co that were applied in the current analysis to further examine dose–response relationships based on studies of oral Co therapy for anemia and oral exposures among the Co beer drinkers. Recent human volunteer studies of Co supplement intake by Tvermoes et al. (2013b) found the biokinetic model to be reasonably accurate for adult males. Finley et al. (2012a) reported that, in healthy individuals, endocrine (thyroid effects) and hematological (polycythemia) effects were the most sensitive responses to Co exposure, and that these responses were unlikely to occur at blood Co concentrations below ~300 μg/L (Finley et al., 2012a). As discussed in detail in this review, it is possible that effects may occur at lower blood Co concentrations in certain susceptible individuals due to an increase in free Co(II) related to rare combinations of underlying disease states and severe perturbations in Co kinetics. These factors may have influenced individual effect levels reported in the various case series examined to date (Tables 4, 6 and 10).

Several disease states can result in clinically severe and sustained hypoalbuminemia (e.g., malnutrition, alcoholism and renal failure) and/or damage to albumin that may impair the Co(II)-binding capacity of human blood. With respect to the latter, IMA can dramatically increase in some individuals as a result of liver disease, kidney failure, ischemic shock, sepsis and diabetes, depending on the severity of the disease. To our knowledge, there are no studies that have actually measured or shown reduced Co–albumin binding *in vivo* in individuals with hypoalbuminemia and/or elevated IMA levels, but we believe these conditions are the most plausible explanations for the enhanced susceptibility to Co-related effects that have been observed in certain individuals or susceptible groups. A subset of the Co beer drinkers suffered from severe cardiomyopathy, yet blood Co concentrations were only 15–180 μg/L (Table 7). According to Finley et al. (2012a), these blood Co concentrations are too low to be associated with adverse effects in healthy individuals, much less destruction of heart muscle tissue. We believe the cardiomyopathy occurred in part because this cohort of affected individuals had elevated free Co(II) ion concentrations due to severe malnourishment (potentially leading to enhanced Co absorption and decreased albumin production) and underlying alcoholic liver and heart disease that left them with severely depleted Co-binding capacity and plausible pre-existing myocardial degeneration.

Similar rationales underlie the susceptibility for the less severe and apparently reversible adverse consequence of high Co doses (polycythemia, hypothyroidism and less frequent neurological effects) that were observed in some individuals with severe forms of renal disease, sepsis and sickle cell anemia who received Co therapy for anemia. Co is no longer used as a beer foam stabilizer or for anemia therapy; however, Co is sold over the counter as a dietary supplement. The doses associated with current Co supplements are generally 1 mg Co/d or less, which is similar to the safe doses proposed by the UK Expert Group on Vitamins and Minerals and by Finley et al. (2012b).

We believe the existing clinical data provide evidence of little to no-risk of Co-related systemic health risk in patients

Table 11. Co speciation of subjects with elevated serum Co: total Co by acid digestion and separate analysis for large (albumin-bound) and small molecular Co (free Co(II) and <1 kDa complexes) according to Kerger et al. (2013a).

Serum code	Analysis	Large molecular Co species	Small molecular Co species	Sum of Co species	Total Co by digestion	Recovery*
Subject 1	Concentration (μg/L)	11.5	0.544	12.1	12.8	94.5%
	Percent of sum of Co	95.5%	4.5%			
Subject 2	Concentration (μg/L)	4.34	0.314	4.65	4.51	103.1%
	Percent of sum of Co	93.2%	6.8%			
Subject 3	Concentration (μg/L)	4.13	0.284	4.41	4.93	89.6%
	Percent of sum of Co	93.6%	6.4%			
Subject 4	Concentration (μg/L)	44.4	3.20	47.6	49.8	95.6%
	Percent of sum of Co	93.3%	6.7%			
Subject 5	Concentration (μg/L)	24.8	1.05	25.8	27.0	95.5%
	Percent of sum of Co	95.9%	4.1%			
Average	Percent of sum of Co	94.3%	5.7%			95.6%
Std. Dev.		1.3%	1.3%			4.8%

Recovery is calculated at 100(Sum of Co Species)/(Total Co by Digestion).

with well-functioning implants. Specifically, a vast majority of the published blood Co concentrations in hip implant patients are below 10 μg/L (Antoniou et al., 2008; Back et al., 2005; Clarke et al., 2003; De Smet et al., 2008; Engh Jr et al., 2009; MacDonald, 2004; Vendittoli et al., 2007; Walter et al., 2008). These values are well below the blood Co no-effect levels presented in Figures 2–5 and are comparable to blood Co concentrations in individuals taking over-the-counter Co supplements (Tvermoes et al., 2013a,b). To our knowledge, there have been no reports that clearly support Co-related health effects occurring in patients with well functioning implants and lower blood Co concentrations (e.g., <10 μg/L).

As discussed earlier, there have been some reports of highly elevated blood Co concentrations and clinical effects in patients with failing implants. These case reports generally involve blood Co concentrations above 400 μg/L (Table 10) and, in some cases, the values exceed the observed effect levels in Figures 2–5. Adverse clinical effects typically resolved following implant removal (and subsequent decrease in Co concentrations), suggesting that Co was at least partially responsible for the observed effects; however, controlled Co dosing studies appear to conflict with some of these anecdotal case reports. Our findings suggest that there is no sound scientific basis for removing an implant from a healthy patient (due to the presence of Co) with ''typical'' blood Co concentrations (e.g., 1–10 μg/L).

Clinical chemistry tools for research on cobalt kinetics and susceptibility

This review provides several lines of evidence suggesting that apparent dose–response anomalies for systemic Co effects are related to a shift in equilibrium binding which favors sustained elevations in free Co(II) ion concentrations in the blood. Co(II) ions are the active biological species for understanding adverse clinical effects because these ions are small enough to penetrate cellular ion channels and it is the divalent cations with greater aqueous stability (not Co(III) or Co(0)) that interact with receptors (e.g., HIF receptors) and biomolecules (e.g., Co binding to lipoic acid and thereby interrupting the citric acid cycle) to induce Co effects. When Co(II) is strongly bound to large proteins (e.g., albumin-bound Co) and perhaps also to certain small molecular complexes (e.g., Co-lipoic acid) it cannot readily enter cells and/or trigger the events that lead to adverse effects. Although, further research is needed to define the magnitude and duration of elevated free Co(II) concentrations that can produce clinically important injury or disease, we suggest that Co speciation in serum will be an effective tool for studying these shifts in equilibrium binding and associated susceptibility differences between individuals.

The recently developed Co speciation assay for the direct analysis of human serum samples (Kerger et al., 2013a) may provide some perspective on individual susceptibility to equilibrium binding shifts favoring free Co(II). For example, Table 11 provides Co speciation data on five subjects with elevated serum Co concentrations, indicating that an average of 94.3% ± 1.3% of the Co is found in the large molecular (albumin-bound) fraction (Kerger et al., 2013a). Similarly, Figure 14 provides Co speciation data for a healthy adult female volunteer who participated in a 90-d study of $CoCl_2$ supplement ingestion at ~1 mg Co/d (Kerger et al., 2013b). This individual had an average of 95.7% ± 2.3% in the large molecular Co fraction, and the average across 12 participants was 95.7% ± 1.6% (Kerger et al., 2013b). Thus, it appears that in healthy adults with serum Co concentrations up to 146 μg/L, the albumin-Co fraction constitutes 94%–96% of the total Co on average. The residual fraction of small molecular Co (e.g., 4%–6% fraction of serum Co on average) would contain both free Co(II) ions and smaller Co-organic complexes (<1 kDa) that are likely to be a more accurate dose metric for predicting systemic toxic effects than the total serum Co concentrations that have been relied upon to date.

The Co speciation method also provides an opportunity to measure the albumin–Co-binding capacity in any person. The partitioning and specific Co binding to serum albumin after spiking $CoCl_2$ in human serum at 2500 μg/L is illustrated in Figure 15, showing ~90% in the large molecular Co fraction even at this extremely high serum Co concentration (Kerger et al., 2013a). These findings demonstrate the considerable capacity of blood proteins to bind Co in healthy individuals.

ACB capacity is known to be influenced by both the quantity and the quality of serum albumin, with diseases causing large increases in IMA potentially affecting the quantitative result. Recall that the clinical assay for IMA

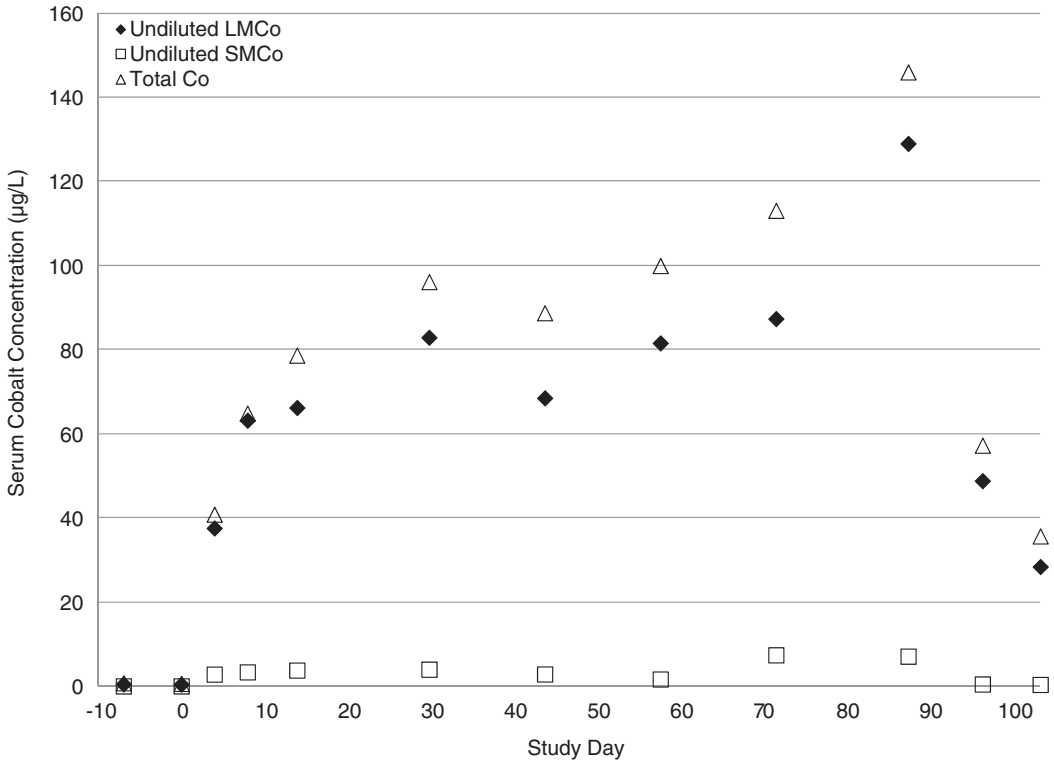

Figure 14. Measurement of total Co and Co speciation (large [LMCo] and small [SMCo] molecular species) in an adult female ingesting ~1 mg Co/d for approximately 90 d (Kerger et al., 2013b). Dosing is from day 1 to 90 d. Pre-dose samples (before dosing started) are represented as the data points at day −10 and 0. One- and two-week post-dose samples (after dosing stopped) are presented as the two data points between day 90 and 100.

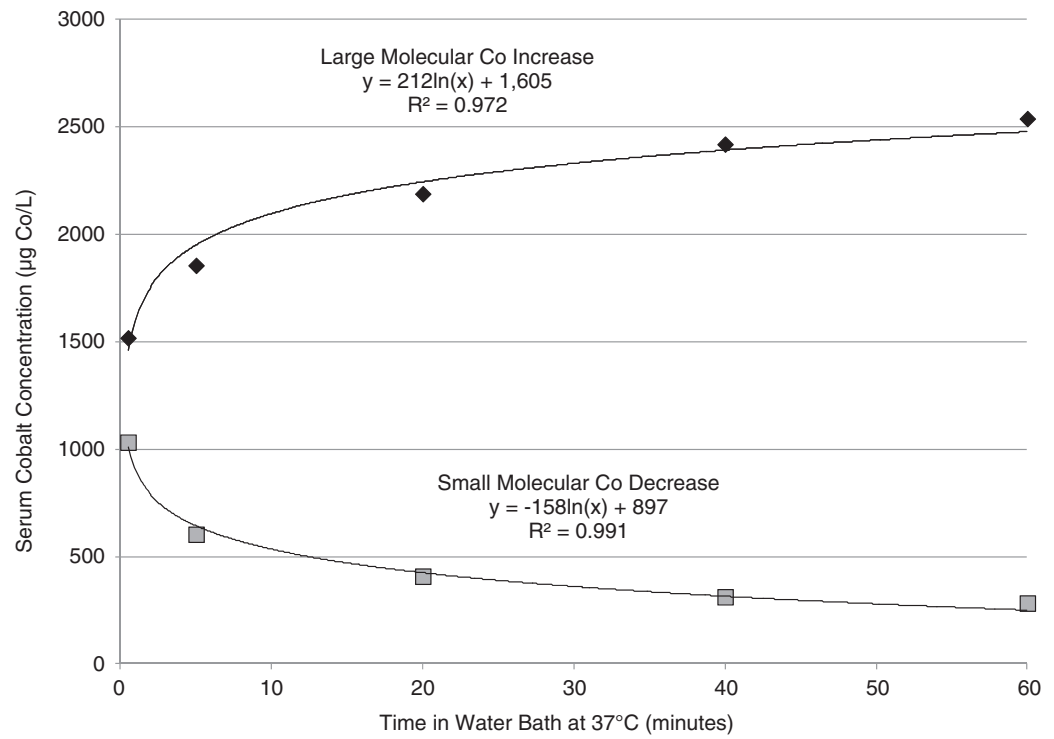

Figure 15. Human serum Co speciation after adding 2500 µg Co/L as $CoCl_2$: concentration versus time in water bath at 37 °C (Kerger et al., 2013a).

(Bar-Or et al., 2000) is defined by reduced Co–albumin binding. Reduced ACB in this assay may be a result of low albumin levels (hypoalbuminemia) and/or a high fraction of damaged albumin or IMA. Individuals who do not have hypoalbuminemia, yet demonstrate reduced ACB in this assay, are likely to have increased IMA. A method for speciating free Co(II) concentrations in serum is also under development (Kerger et al., 2013a). Further characterization of the kinetics of Co(II) ions in animal studies and in clinical settings should

provide greater insights on dose–response relationships and individual susceptibility for systemic Co effects.

Risk-based health monitoring guidelines for blood cobalt concentrations in hip prosthesis patients

Guidance values for blood Co concentrations have been suggested by some organizations for hip implant patients, and these values are well below the no-effect levels identified by Finley et al. (2012a). However, it is critical to recognize that these suggested guidance values are not necessarily health risk-based. For example, the UK Medicines and Healthcare Products Regulatory Agency (MHRA) proposed a blood Co criterion of 7 μg Co/L for a follow-up of both symptomatic and asymptomatic MoM hip implant patients. The agency has stated that symptomatic patients with ''[b]lood metal ion level >7 ppb [μg/L] indicates potential for soft tissue reaction,'' and has recommended metal artifact reduction sequence magnetic resonance imaging (MARS MRI) or ultrasound imaging, as well as, additional blood metal testing 3 months later (MHRA, 2012, p. 6). This blood concentration of Co has been embraced as an indicator of the degree of wear that is occurring at the implant and was not intended to suggest an increased risk of developing systemic effects. Indeed, the basis for the 7 μg/L guideline was a statistical interpretation of blood-metal concentrations in hip implant patients where wear was occurring. In contrast, the Mayo Clinic has suggested that Co-related effects might occur at serum Co-concentrations of 5 μg/L and greater if ''cobalt is ingested'' (Mayo Clinic, 2012). However, no information was presented on the derivation of this value.

Also, the FDA ''currently believes there is insufficient evidence to correlate the presence of localized lesions, clinical outcomes, and/or the need for revision with specific metal ion levels for individual patients'' (USFDA, 2012, 2013). Similarly, the Therapeutic Goods Administration (TGA), Australia's regulatory agency for therapeutic goods, has stated that ''[a]t this point in time there is no accepted level of cobalt or chromium in the blood that is associated with adverse health impacts. Systemic toxicity arising from cobalt or chromium leaching from MoM hip implants has not been clearly demonstrated, but cannot be excluded'' (TGA, 2012).

Mayo Clinic notes in its publication, Communique, that ''Clinically important implant wear is indicated when serum chromium exceeds 15 ng/mL and cobalt exceeds 10 ng/mL; these symptomatic patients are likely to have significant implant deterioration.'' They also note that ''Elevated chromium and cobalt concentrations may indicate implant wear, but they are not considered a health hazard'' (Mayo Clinic, 2012). Further, the Mayo Clinic notes that there are no large case number reports associating high circulating serum cobalt with toxicity'' (Mayo Medical Labs, 2013).

The dose–response anomaly identified in studies of the affected Co beer drinkers who developed fatal cardiomyopathy at exceptionally low ingested doses (while others did not) requires careful consideration. As shown in Figure 5, myocardial changes or disease were not identified in occupational groups or hypertension patients at blood Co concentrations up to 38 μg/L. Co beer drinkers represent a uniquely susceptible group with the dose–response relationship being influenced by severe malnutrition and underlying chronic liver and heart disease from chronic, severe alcoholism. The noted effect levels in Table 6 are as low as 0.04–0.07 mg Co/kg-d, far lower than those identified for other susceptible patient groups. Due to malnourishment, Co absorption was probably at the high end for affected beer drinkers (Table 7), corresponding to steady-state blood Co concentrations of 15 to 180 μg/L based on the Unice et al. (2012) biokinetic model at 15% absorption and 50% absorption. Indeed, the affected Co beer drinkers exhibited lethal cardiomyopathy at blood Co concentrations at least an order of magnitude lower than those estimated or measured in patients receiving Co therapy for anemia who exhibited no adverse Co effects, or the few who exhibited generally reversible effects on thyroid function and vision/hearing impairment. Thus, there must be unique susceptibility factors affecting Co kinetics/toxicity in the affected Co beer drinkers, making them not comparable to other studied individuals or groups.

Another topic that deserves special attention is understanding why some persons develop reversible optic or auditory neuropathy at the low end of the therapeutic dose range (e.g., at blood Co concentrations between 420 and 700 μg/L). These effects have been reported primarily among anemic kidney failure patients on dialysis with measured serum Co concentrations at the end of dosing ranging from 420 to 2100 μg/L (560, 600, 2100 and 420 μg/L); peak Co serum concentrations reported during dosing ranged from 820 to 2100 μg/L (820, 1620, 2100 and 940 μg/L) (Bowie & Hurley, 1975; Duckham & Lee, 1976). Although one case of reversible vision and hearing impairment was reported for an individual with occupational Co exposure and a blood Co concentration of 234 μg/L, depletion of blood Co from the end of his last exposure to the date of measurement (a 3-month period) indicates a plausible peak dose at 2900 μg/L (Meecham & Humphrey, 1991). Considered together, these data suggest that the rare occurrence of neuropathy responses is isolated to higher systemic blood Co concentrations; usually higher than 400 μg/L.

The most rigorous dose–response analysis for the lowest dose effects of Co (polycythemia and reduced thyroidal iodine uptake) was conducted by Jaimet & Thode (1955), who administered multiple dose regimens to 18 children and identified no adverse responses at 1.8 mg Co/kg-d (corresponding to blood Co concentrations of 650–1500 μg/L) and found reversible reductions in thyroidal iodine uptake in two of the 18 children at 2.7 mg Co/kg-d (corresponding to blood Co concentrations of 920–2140 μg/L). Other studies confirmed that no reduction in thyroidal iodine uptake was observed in anemic adults treated with 1 mg Co/kg-d (corresponding to blood Co concentrations of 300–700 μg/L) or in anemic adults on dialysis for kidney failure at measured blood concentrations of 220–2100 μg/L (Bowie & Hurley, 1975). Thus, there is a reasonably robust basis across the available reports on susceptible groups or individuals to surmise that 300 μg/L can be used as a point of departure (POD) (e.g., the apparent threshold dose) for reversible effects of Co on polycythemia and thyroidal iodine uptake.

We suggest that the observation of reversible polycythemia in non-anemic adult males at 0.97 mg Co/kg-d, corresponding to blood Co concentrations at or above 300 μg/L, can serve as

a POD for developing risk-based values to help make decisions about whether to monitor hip prosthesis patients to test for some systemic effects. In risk assessment, the POD is the dose from which one can estimate the dose of a chemical that is not likely to produce adverse effects in the general population. The blood concentration of about 300 μg/L corresponds to reversible and clinically non-serious changes in blood counts or thyroidal iodine uptake across two independent studies (Davis & Fields, 1958; Roche & Layrisse, 1956), and is comparable to the lowest POD across Co studies in both humans and animals [300 μg/L] reported by Finley et al. (2012a,b). Higher Co doses were generally well tolerated by anemia patients, with only one report of reversible reduction in thyroidal iodine uptake at a lower dose (0.54 mg Co/kg-d, corresponding to blood Co concentrations of about 150–350 μg/L; Paley et al., 1958). Anemic patients, including young children and pregnant women, have been noted to take sustained daily doses of 0.53–1.8 mg Co/kg-d for months without adverse clinical effects. The actual blood concentration that may increase the risk of polycythemia after 2–3 weeks of exposure may be greater than 300 μg/L because this value was model-estimated using a very low level of Co absorption (15%).

Based on currently available data, it might be useful to monitor implant patients for signs of hypothyroidism and polycythemia starting at blood or serum Co concentrations above 100 μg/L. This concentration is derived by applying an uncertainty factor of 3 to the 300 μg/L POD, which should adequately account for inter-individual variability, since the data were collected across a relatively diverse set of exposed patients and volunteers. Closer follow-up of patients who also exhibit chronic disease states leading to clinically important hypoalbuminemia and/or severe IMA elevations should be considered.

Proposed areas for future research

Since the systemic bioavailability and toxicity of Co compounds is apparently tied to concentrations of free Co(II) ions in blood and tissues, it would be particularly helpful to conduct research that evaluates the ionic Co concentrations rather than total Co concentrations in blood. With this information, a dose–response curve for free Co(II) could be identified, which could be used for setting future regulatory limits. Current regulatory efforts and medical guidelines for Co blood concentrations in joint prosthesis patients do not take this mode of action into account, and health concerns regarding Co–Cr implants should almost certainly be based on the free Co(II) ion concentrations in blood to characterize thresholds for various adverse effects.

The results of studies of children and adults treated in the 1950s and 1960s provide confidence that, for the vast majority of implant patients who may have implants installed after age 40, no systemic effects due to Co–Cr alloys with typical wear corresponding to total Co blood concentrations below 10 μg/L should be expected. However, there is a limited database of studies examining chronic systemic Co exposures, and, therefore, further research is needed to address uncertainties associated with these data gaps. We identified only three case reports prior to 2010 in the literature of systemic health effects associated with MoP or MoM implants resulting from excessive wear of the implant due to residual ceramic shards from a previous ceramic prosthesis that had fractured; blood Co concentrations associated with the reported effects ranged from approximately 400 to 625 μg/L (Oldenburg et al., 2009; Rizzetti et al., 2009; Steens et al., 2006). Case reports are, by definition, hypothesis generating and usually not sufficient to conclude there is a cause/effect relationship.

Specifically, further research is warranted of the metal ion-binding capacity and free Co(II) ion concentration in persons with chronic systemic exposure to Co from dietary supplements or Co–Cr hip implants in order to augment the available database on Co mode of action and dose–response relationships in humans. From a pharmacokinetic and mechanistic perspective, the currently available evidence on apparently susceptible groups indicates that the concentration of free Co(II) ions may be the best dose metric to understand dose–response relationships in individuals with elevated total Co blood concentrations.

Declaration of interest

All the authors are employed by ChemRisk, a consulting firm that provides scientific advice to the government, corporations, law firms and various scientific/professional organizations. ChemRisk has been engaged by DePuy Orthopaedics, Inc., a manufacturer of prosthetic devices some of which contain cobalt. This paper was prepared and written exclusively by the authors, without review or comment by DePuy employees or counsel. It is likely that this work will be relied upon in medical research, nutrition research and litigation. Some of the authors may be called upon to serve as expert witnesses. Funding for the paper was provided by DePuy.

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Dose-Response Relationships For Blood Cobalt Concentrations and Health Effects: A Review of the Literature and Application of a Biokinetic Model

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DOSE-RESPONSE RELATIONSHIPS FOR BLOOD COBALT CONCENTRATIONS AND HEALTH EFFECTS: A REVIEW OF THE LITERATURE AND APPLICATION OF A BIOKINETIC MODEL

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Cobalt (Co) is an essential component of vitamin B_{12}. As with all metals, at sufficiently high doses, Co may exert detrimental effects on different organ systems, and adverse responses have been observed in animals, patients undergoing Co therapy, and workers exposed to respirable Co particulates. Although blood Co concentrations are postulated to be the most accurate indicator of ongoing Co exposure, little is known regarding the dose-response relationships between blood Co concentrations and adverse health effects in various organ systems. In this analysis, the animal toxicology and epidemiology literature were evaluated to identify blood Co concentrations at which effects have, and have not, been reported. Where necessary, a biokinetic model was used to convert oral doses to blood Co concentrations. Our results indicated that blood Co concentrations of 300 μg/L and less have not been associated with adverse responses of any type in humans. Concentrations of 300 μg/L and higher were associated with certain hematological and reversible endocrine responses, including polycythemia and reduced iodide uptake. Blood Co concentrations of 700–800 μg Co/L and higher may pose a risk of more serious neurological, reproductive, or cardiac effects. These blood concentrations should be useful to clinicians and toxicologists who are attempting to interpret blood Co concentrations in exposed individuals.

Cobalt (Co) is a component of cyanocobalamin, an essential vitamin (vitamin B_{12}) required for producing red blood cells (RBC) and preventing pernicious anemia (Barceloux, 1999). In the general population, diet is the main source of Co exposure, and dietary Co intake in the United States has been estimated to range between 5 and 40 μg Co/d, with the highest Co concentrations found in fish, green leafy vegetables, and fresh cereals (World Health Organization [WHO], 2006; Hokin et al., 2004b, 2004a). The mean serum Co concentration in the general population was reported to be 0.19 μg/L, with 95% of individuals having concentrations below 0.41 μg/L (Alimonti et al., 2005).

It is well established that oral exposure to Co might yield desired biological responses at therapeutic doses (e.g., greater than 1 mg/kg-day), but that some adverse effects may also occur in individuals exposed to similar or higher doses. For example, in the 1950s, oral Co therapy was often prescribed for anemic patients, including children and pregnant women, at doses ranging from 6 to 150 mg/d (Holly, 1955a; Holly, 1955b; Booth and Montgomery, 1956; Davis and Fields, 1958). At that time, consuming Co was known to stimulate the

All of the authors are employed by ChemRisk, a consulting firm that provides scientific advice to the government, corporations, law firms, and various scientific/professional organizations. ChemRisk has been engaged by DePuy Orthopaedics, Inc., a manufacturer of prosthetic devices some of which contain cobalt. This article was prepared and written exclusively by the authors without review or comment by employees or counsel for DePuy. It is likely that this work will be relied upon in medical research, nutrition research, and litigation. Some of the authors may be called upon to serve as expert witnesses. Funding for the paper was primarily provided by DePuy.

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production of hemoglobin and RBC. However, cases involving the appearance of goiters and other thyroid-related effects in a small fraction of these patients were also noted (Kriss et al., 1955; Gross et al., 1955). Thus, Co treatment for anemia was eventually replaced with synthetic erythropoietin and other drugs.

In the past, Co was sometimes added to beer to serve as a foam stabilizer. Interestingly, in the 1960s, a small cohort of heavy beer drinkers (up to dozens of beers per day) developed severe cardiomyopathy after consuming Co doses of 5–10 mg/d for up to 12 months. Malnutrition was shown to have largely contributed to the cardiomyopathic effects (Kesteloot et al., 1968). The role of Co was difficult to differentiate from effects of chronic alcohol ingestion.

Currently, oral Co therapy is still used to treat hyperexcretion of estrogens that sometimes occurs during female hormone replacement therapy (Wright, 2005). In addition, many over-the-counter Co supplements are sold (e.g., Mineral Life Ionic Mineral Supplement, Colorado Springs, CO; Mother Earth Minerals, Ogden, UT; and Treasure of the Earth Liquid Ionic Angstrom Cobalt, Silt, CO) at recommended doses up to 1 mg/d. These supplement manufacturers claim that Co aids fat and carbohydrate metabolism, protein synthesis, and RBC production, as well as myelin sheath repair in the central nervous system (DRN, 2012; DRI, 2011; MEMI, 2011). Many individuals also supplement their diets with large doses of Co-containing vitamin B_{12}. For example, The Vegan Society (VS), the Vegetarian Resource Group (VRG), and the Physicians Committee for Responsible Medicine (PCRM), among others, recommend that vegans either consistently eat foods fortified with B_{12} or take a daily or weekly B_{12} supplement (VS, 2012; VRG, 2012; PCRM, 2012).

Some agencies have established health-based guidance values for dietary Co supplementation. The United Kingdom Expert Group on Vitamins and Minerals, for example, concluded that supplementing with 1400 μg Co/d was unlikely to produce adverse health effects in adults (EGVM, 2003). The European Food Safety Authority (EFSA) suggested that a dose of 600 μg Co/d might be considered safe (EFSA, 2009). Recently Finley et al. (2012) suggested that the ingestion of 0.03 mg/kg-d (2100 μg/d for a 70-kg adult) should be safe throughout a lifetime of exposure for healthy individuals. These doses are well beyond the typical dietary intake of 5–40 μg/d.

In spite of the known beneficial and adverse responses associated with various doses of Co, little is understood regarding the dose-response relationships between tissue Co levels and systemic organ effects. Although blood Co concentrations are postulated to be the most reliable measure of ongoing Co exposure, few studies have examined Co-related systemic health effects as a function of the concentration of Co in blood. To address this data gap, Unice et al. (2012) recently developed a biokinetic model that provides estimates of blood Co concentration as a function of oral Co dose. This model was based on the Co biokinetic model originally reported by Leggett (2008), as well as on the standard human alimentary tract model used to assess oral absorption (ICRP, 2006; Leggett, 2008). Our recent studies with human volunteers indicate the model is reasonably accurate at predicting blood Co concentrations following oral dosing of adult human males with known amounts of Co (Tvermoes et al., 2012).

In this paper, the animal toxicology and epidemiology literature were examined to identify oral Co doses at which systemic organ effects have, and have not, been reported. These doses, in conjunction with the biokinetic Co model, were then used to estimate blood Co concentrations at which different adverse health effects are not likely to occur, as well as those metal concentrations that may pose a specific health risk. The blood concentrations identified may be used as biological indices to evaluate risks to individuals who may have elevated blood Co concentrations due to (1) occupational Co exposures, (2) prescribed medical Co therapies, (3) voluntary ingestion of vitamin B_{12} and other Co-containing supplements, (4) ingestion of Co-contaminated water and other environmental media, or (5) the presence of Co-containing prosthetics and other medical

devices. The aim of this review was to correlate potential adverse health risks with blood Co concentrations in exposed individuals and identify factors that may enhance Co-induced toxicity in susceptible populations.

LITERATURE SEARCH AND IDENTIFICATION OF RELEVANT STUDIES

A primary literature search was conducted to identify human and animal studies that evaluated health effects following Co exposure. The Agency for Toxic Substances and Disease Registry (ATSDR) toxicological profile for Co and the U.S. Environmental Protection Agency (EPA) Office of Research and Development's National Center for Environmental Assessment's (NCEA) provisional peer-reviewed toxicity value (PPRTV) for Co were utilized as secondary literature sources. The literature search was conducted in PubMed using the following search terms: cobalt AND (toxicity OR health effects OR cardiotoxicity OR hematological OR endocrine OR immunological OR reproductive OR testicular effects OR neurological).

The literature search was two-tiered. In phase I, all articles were initially gathered that evaluated Co exposures in humans or animals, regardless of exposure pathway; however, in vitro studies were not considered. Studies of hip implant patients were not included because of potential confounding factors such as drug therapy and preexisting conditions. The phase I search yielded approximately 500 relevant articles. These papers were evaluated to identify those systemic health effects that were either (1) consistently observed in humans or (2) consistently observed in animals with some corroborating evidence in humans. These were classified as the "target endpoints."

For the purposes of determining the blood Co dose-response relationship for these target endpoints, we considered any non-acute animal or human inhalation, dermal, or intravenous (iv) study or case report which (1) evaluated a target endpoint and (2) included blood Co measurements. Any nonacute animal or human oral study that evaluated one or more of the target endpoints following ingestion Co in any medium including water, food, or gavage was also considered. Acute oral studies that did not report dosing information and/or those that lacked sufficient information for statistical analysis or determination of an effect or no-effect level were excluded. Studies involving infants were also excluded. Each study was assessed to determine whether the results may have been confounded by chemical coexposures or other factors. In our view, all relevant studies were collected and properly classified.

For each study, "no-effect" and/or "effect" levels were identified, if possible. For the purposes of this analysis, in each study the "no-effect" level is the highest oral Co dose or blood Co concentration at which no effect occurred, while the "effect" level is the lowest oral Co dose or blood Co concentration at which a Co-related effect was noted. Individual case reports were not used to identify effect and no-effect levels unless blood Co concentrations measured during the Co exposures were reported. For oral dosing studies in which blood Co concentrations were not reported, the blood Co concentrations associated with the effect and no-effect doses were estimated using a biokinetic model (Unice et al., 2012). Studies with potential confounders such as coexposure to other chemicals that reported no apparent effects were assessed for possible no-effect levels. Studies with potential significant confounders that reported the occurrence of effects were not included as possible sources of effect levels. In studies with multiple exposure durations, only the results from the longest exposure period were considered.

In the calculations, where necessary, a default weight of 70 or 75 kg (for pregnant women) for body weight was utilized for adult human studies unless a dose was reported as mg Co/kg-d. Further, when appropriate, default assumptions were made for ingestion rates and weight in rats or mice (e.g., 0.139 L/d for water intake in a male Sprague-Dawley rat) that were consistent with the Recommendation for and

Documentation of Biological Values for Use in Risk Assessment used by the U.S EPA to determine the daily dose (U.S. EPA, 1988, 2011a). To determine a human equivalent dose (HED) from an animal dose, a dosimetric adjustment factor applied for toxicokinetic differences (e.g., 4.16 for rats) was utilized as suggested by the U.S. EPA (2011b). When not reported, the salt of Co assumed for "cobalt chloride" was $CoCl_2$ with a molecular weight of 129.8 g.

BIOKINETIC MODEL

A biokinetic model was used to estimate blood Co concentrations from ingestion of known doses of Co (Unice et al., 2012). Figure 1 shows the predicted blood Co concentrations for a 70-kg adult ingesting 1 mg Co/d for a year. The estimated blood concentrations are 24-h averages and based on an exposure time equal to the shortest exposure duration reported in each study. A total blood volume of 5.3 L and a urinary excretion rate of 1.5 L/d (which corresponds to a 70-kg adult) were assumed in this model (Leggett and Williams, 1991; McNally et al., 2011). Gastrointestinal tract (GIT) absorption of Co is reported to be highly variable and depends on the dose, gender, delivery vehicle, and solubility of the ingested Co compound. Additional factors, such as time since last meal and iron (Fe) status, are also known to alter GIT absorption of Co (Engel et al., 1967; Harp and Scoular, 1952; Leggett, 2008; Paley and Sussman, 1963; Smith et al., 1972; Sorbie et al., 1971; Valberg et al., 1969). As indicated by Unice et al. (2012), GIT absorption rates in humans typically range from 15 to 35%. A GIT absorption rate of 15% was assumed when estimating blood Co concentrations from animal and human studies (Leggett, 2008; Smith et al., 1972).

IDENTIFICATION OF TARGET ENDPOINTS

The target endpoints considered in this analysis were: hematological, cardiovascular, endocrine, neurological, and reproductive. Hematological and endocrine responses may occur in both animals and humans exposed to sufficiently high doses of Co. Specifically, numerous clinical studies reported

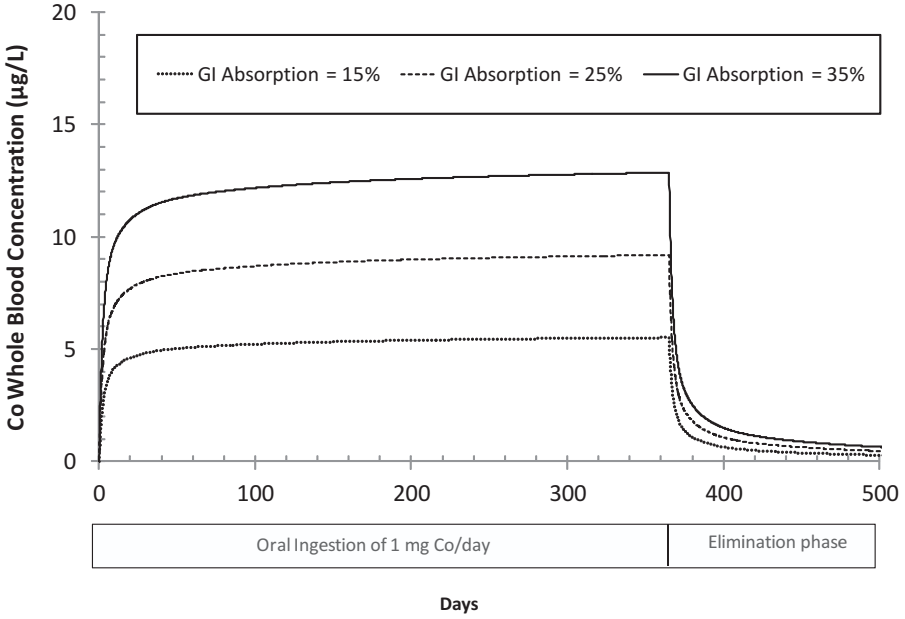

FIGURE 1. Illustrative example of the uptake, time to steady state, and elimination of Co if ingested for 365 days (assuming 15, 25 and 35% absorption in the GI tract). The dose was 1 mg/day of Co and the values shown are based on continuous dosing for a 70 kg male. The figure is based on the application of the Unice et al (2012) model.

polycythemia and reduced iodide uptake (and goiter development) in healthy or anemic humans therapeutically dosed with Co. Polycythemia and reduced iodide uptake were also demonstrated in several animal studies. For the purposes of this analysis, if standard indicators of polycythemia such as increases in RBC counts or volume, hemoglobin, or hematocrit were reported in Co-exposed animals or humans, these were considered to be a Co-related response unless confounding coexposures to other chemicals suggested otherwise. Similarly, if standard measures of hypothyroidism including decreases in triiodothyronine (T3) uptake or circulating thyroxine (T4) levels, increased thyroid-stimulating hormone (TSH) levels, and/or the appearance of a goiter occurred in Co-exposed humans or animals, these were considered as possible evidence of Co-induced hypothyroidism. Because the hematological and endocrine endpoints are more accurately defined as biological responses that are not necessarily indicative of a health risk, as opposed to harmful (and possibly irreversible) adverse health effects, the no-effect and effect levels for these endpoints are termed "no-observed-effect levels" (NOELs) and "lowest-observed-effect levels" (LOELs).

While "beer drinker's cardiomyopathy" is sometimes taken as evidence of Co-related effects in humans, the syndrome also shares several similarities with alcoholic cardiomyopathy (Richardson et al., 1986; Barceloux, 1999), and poor diet was also shown to be a significant factor in the disease progression in this cohort (Kesteloot et al., 1968). Nonetheless, case reports of occupational exposures to Co have been associated with cardiomyopathy (Jarvis et al., 1992; Kennedy et al., 1981), and adverse cardiovascular effects were observed in animals exposed to high concentrations of Co via oral and inhalation routes. Therefore, cardiac effects measured as increased serum levels of myocardial creatine kinase, pericardial effusion, multifocal myocytolysis, and other typical metrics of cardiac damage were included as a target organ endpoint in this analysis.

There is little to no information to suggest that Co produces adverse reproductive effects in humans, but some animal studies reported such effects, and, therefore, this endpoint was included in this analysis. Similarly, there are case reports describing hearing and vision disturbances in patients on Co therapy and therefore neurological disturbances were considered in our analysis. Because some cardiac, neurological, and reproductive endpoints involve adverse (and perhaps irreversible) effects (as opposed to biological responses, which may not be indicative of risk, and are often reversible), the no-effect and effect levels for these endpoints are termed "no-observed-adverse-effect levels" (NOAELs) and "lowest-observed-adverse-effect levels" (LOAELs).

ESTIMATED Co BLOOD CONCENTRATIONS ASSOCIATED WITH HEMATOLOGICAL RESPONSES

Cobalt was shown to stimulate erythropoiesis in human and animal studies above certain doses. As a result, Co was used therapeutically in the past to treat anemic patients, and is currently taken by (1) some athletes to increase the oxygen-loading capacity of the blood (Lippi et al., 2006; Jelkmann and Lundby, 2011), (2) some women suffering from the hyperexcretion of estrogens during female hormone replacement therapy (Wright, 2005), and (3) others who take vitamin B_{12} and Co supplements for perceived health benefits.

Some of the human studies that reported hematological responses following Co exposure were not considered suitable for this analysis because of confounding factors or other reasons. Specifically, many of the relatively older studies that noted polycythemia and other hematological responses at doses ranging from 0.5 to 10 mg Co/kg-d lacked sufficient information for statistical analysis or NOEL/LOEL determination (Stanley et al., 1947; Murdock, 1959). Taylor et al. (1977) reported that polycythemia occurred in 8 patients dosed at 25–50 mg $CoCl_2$/d (0.16–0.32 mg Co/kg-d); however, this study involved anephric patients who were

undergoing dialysis. Curtis et al. (1976) established that kidney dysfunction and dialysis lead to poor Co clearance from the bloodstream and therefore it is not possible to accurately estimate blood Co concentrations with the biokinetic model in the patients from Taylor et al. (1977) because the model assumes the presence of normal, healthy kidneys. In reality, this model would likely far underestimate actual blood Co concentrations.

Other studies evaluated hematological responses in humans at much higher Co doses. Davis and Fields (1958) exposed 6 healthy men aged 20–47 yr to a 2% $CoCl_2$ solution for up to 22 d. Five of 6 men received 150 mg $CoCl_2$/d (68 mg Co/d) for the entire exposure period, while the sixth started on 120 mg/d (54 mg Co/d) and later received 150 mg Co/d. At this exposure (0.97 mg Co/kg-d), all six subjects reportedly developed reversible polycythemia where the RBC concentrations increased during dosing and then returned to baseline within a few days post dosing. Although it is unclear whether the numerical increases in RBC and percent hemoglobin levels noted in this study (11 and 21%, respectively) were statistically significant, the 0.97-mg Co/kg-d dose in this study was considered as a LOEL (estimated blood Co concentration of 320 μg/L, as reported in Table 1 and Figure 2). Jaimet and Thode (1955) dosed 15 young children (ages 5–9 yr) with 0.45, 0.90, 1.8, or 2.7 mg Co/kg-d for 10 wk. No change in blood hemoglobin levels was observed at any dose; however, at the highest dose there were interruptions in the dosing schedule, and therefore the 1.8-mg/kg-d dose (estimated blood Co concentration of 650 μg/L) was considered to be the NOEL from this study (Table 1 and Figure 2). Holly (1955b) found no significant alterations in hemoglobin levels following Co administration (0.45–0.6 mg Co/kg-d, midpoint of 0.53 mg Co/kg-d) to 20 pregnant women (estimated midpoint NOEL blood Co concentration of 200 μg/L).

Several studies identified both hematological responses and blood Co concentrations in Co-exposed cohorts. Duckham and Lee (1976) measured blood Co concentrations in 4 dialysis patients (reported as case initials: R.D., R.B., A.T., M.V.) ingesting 0.18 mg Co/kg-d for 4 to 12 wk. It is important to note that because the kidney is a major source of erythropoietin, individuals with kidney disease are often anemic, and Co therapy was historically used to increase RBC counts in dialysis patients. Mean hemoglobin levels were elevated by 16–55%, and blood Co concentrations in these 4 patients during the dosing period ranged from 640 to 1220 μg/L (mean of approximately 900 μg Co/L, shown as a LOAEL in Table 1 and Figure 2). Bowie and Hurley (1975) measured blood Co concentrations in 11 dialysis patients taking 11.3 mg Co for 4 wk, followed by 22.6 mg Co for 4 wk. Mean hematocrit and RBC volumes were significantly increased after 8 wk compared to pre-dosing, and blood Co concentrations at 8 wk ranged from 220 to 2100 μg/L (mean approximately 600 μg/L, shown as a LOEL in Table 1 and Figure 2).

In another study with blood Co measurements, Angerer et al. (1985) found that in 40 foundry workers exposed to airborne Co concentrations of 49 to 1046 $\mu g/m^3$ (workers were employed at the facility for 0.5–36 yr) no effects on "blood cell counts" at Co blood concentrations ranging from 4.9 to 47.9 μg /L occurred. Although no additional details or data regarding hematological effects were offered in the paper, the midpoint of this range (26 μg Co/L) was utilized as a human NOEL for polycythemic effects (Table 1 and Figure 2).

Raffn et al. (1988) evaluated a cohort of porcelain painters exposed to a Co-containing blue dye. Data showed "minor" and "very small" decreases in hematocrit, hemoglobin and mean cell volume and it was concluded that these effects were not related to Co concentrations in the blood as Co exposure normally produces a rise in these parameters. The mean blood Co value of 2.1 μg/L as measured after the painters resumed work following a "work-free period" was taken as a hematological NOEL (Table 1 and Figure 2). Similarly, Swennen et al. (1993) reported a reduction in RBC and hemoglobin levels (hematocrit was unchanged) in workers exposed to Co oxides, salts, and metals at a

TABLE 1. No Observed Effect Level and Effect Level Doses in Humans with the Corresponding Blood Concentrations

Reference	Exposed group	Response evaluated	Response category	NOAEL Dose (mg Co/kg-day)	LOAEL Dose (mg Co/kg-day)	No response observed: estimated or reported blood Co levels (μg/L or ppb)	Response observed: estimated or reported blood Co levels (μg/L or ppb)
Holly, 1955b[a]	20 pregnant women	No hematological alterations	Hematological	0.53	NA	200	
Jaimet and Thode, 1955	18 children (10 male and 8 female)	No change in hemoglobin levels	Hematological	1.8	NA	650	
Davis and Fields 1958	6 healthy adult males	Polycythemia	Hematological	NA	0.97		320
Bowie and Hurley, 1975	11 adult dialysis patients	Increased hematocrit and red blood cell volume	Hematological	NA	0.32		600[b] (110)
Duckham and Lee, 1976	4 adult dialysis patients	Increased hemoglobin levels	Hematological	NA	0.18		900[b] (65)
Angerer et al., 1985	40 foundry workers	No effects on erythropoesis	Hematological	NA	NA	26[b]	
Raffn et al., 1988	46 plate painters	Decreased hematocrit and mean cell volume, no changes in hemoglobin and red blood cells	Hematological	NA	NA	2.1[b]	
Swennen et al., 1993	82 foundry workers	Decreased red blood cells and hemoglobin, no changes in hematocrit	Hematological	NA	NA	12.7[b]	
Lantin et al., 2011	249 foundry workers	No change in red blood cells	Hematological	NA	NA	1[b]	
Jacquet, 1949	Hypertension pateints	No cardiac effects	Cardiac	0.11	NA	38	
Kesteloot et al., 1968	12 beer drinkers	No cardiomyopathy	Cardiac	0.09	NA	34	
Angerer et al., 1985	40 foundry workers	No cardiomyopathy	Cardiac	NA	NA	26[b]	
Raffn et al., 1988	46 plate painters	No differences in electrocardiography	Cardiac	NA	NA	2.1[b]	
Swennen et al., 1993	82 foundry workers	No serum changes in myocardial protein kinase	Cardiac	NA	NA	12.7[b]	
Jaimet and Thode, 1955	18 children (10 male and 8 female)	Decreased iodine uptake	Endocrine	1.8	2.7	650	920
Roche and Layrisse 1956	12 adults (gender not specified)	Decreased iodine uptake	Endocrine	NA	0.97		300
Bowie and Hurley, 1975	11 adult dialysis patients	No changes in serum thyroxine and TSH	Endocrine	0.32	NA	600[b] (110)	
Swennen et al., 1993	82 foundry workers	No changes in T3 uptake, T4, or TSH, decrease in total T3	Endocrine	NA	NA	12.7[b]	
Lantin et al., 2011	249 foundry workers	No changes in serum T3, T4, and TSH	Endocrine	NA	NA	1[b]	
Bowie and Hurley, 1975	12 adult dialysis patients	Reversible hearing loss	Neurological	0.32	0.32	440[b] (110)	1087[b] (110)
Duckham and Lee, 1976	4 adult dialysis patients	No detectable nerve damage or polyneuropathy	Neurological	0.18	NA	900[b] (65)	
Meecham and Humphrey, 1991	1 adult occupationally exposed to Co	Reversible vision and hearing loss	Neurological	NA	NA		234[c] (2900[d])

[a]For pregnant women, a default body weight of 75 kg was assumed (EPA, 2011a).
[b]In these studies, a blood Co concentrations was reported. Estimated blood Co concentrations are in parathenses for these oral ingestion studies.
[c]Reported 3 months after leaving job.
[d]Estimated blood Co concentration for the worker if calculated at the time he left work (using biokinetic model). *All calculations used to estimate blood Co concentrations are provided in the online supplementary materials.

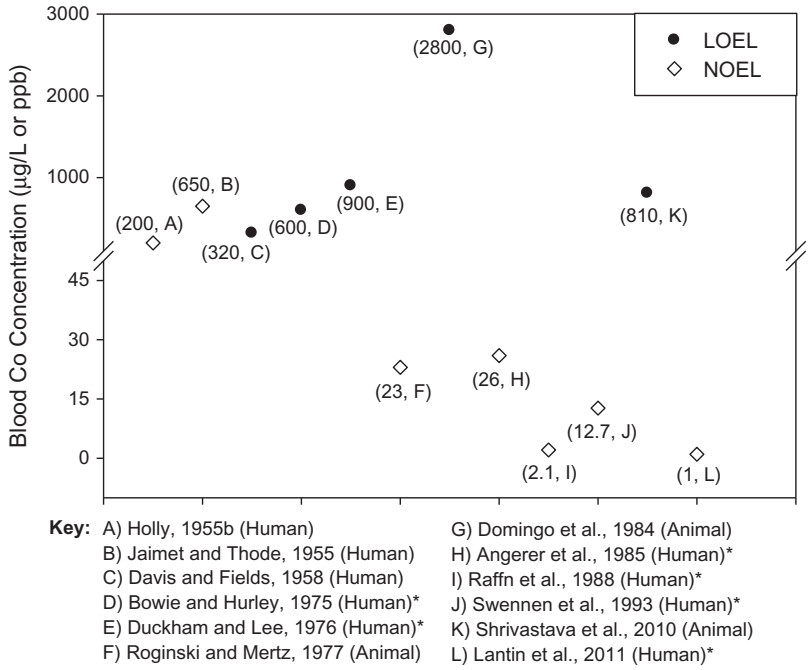

FIGURE 2. Reported or estimated blood Co NOELs and LOELs for hematological effects. *Reported blood Co concentrations are denoted by an asterisk next to the study (*).*

plant in Belgium. Swennen et al. (1993) noted that this finding contrasted with "previous animal experiments suggesting that administration of high doses of Co may cause polycythemia" (p. 840). Because these changes are unlikely to be Co-related, the mean blood level of 12.7 µg/L (measured Friday post shift) was considered as a NOEL (Table 1 and Figure 2). Lantin et al. (2011) evaluated workers from the same plant and found no marked changes in RBC or hemoglobin values (vs. a referent group); the median blood Co level was 1 µg/L, which was considered to be a hematological NOEL (Table 1 and Figure 2).

With respect to animal studies, Domingo et al. (1984) found polycythemia in rats dosed with 31.7 mg Co/kg-d (HED = 7.6 mg Co/kg-d) for 3 mo (estimated blood Co LOEL of 2800 µg/L, as shown in Table 2 and Figure 2). Roginski and Mertz (1977) reported no increase in hemoglobin levels in rats ingesting food containing 3 mg Co/kg for eight weeks. Assuming an ingestion rate of 0.091 mg food/kg-day (EPA, 1988), the Co dose ingested by these animals is approximately 0.27 mg Co/kg-d (HED = 0.065 mg Co/kg-d). The corresponding blood Co concentration estimated using the biokinetic model is 23 µg Co/L (shown as a NOEL in Table 2 and Figure 2). Shrivastava et al. (2010) orally dosed male Sprague-Dawley rats (n = 8 per group) with distilled water or 12.5 mg Co/kg-d for 7 d and reported a statistically significant increase in RBC, hemoglobin, and hematocrit as a result of treatment. Even though Shrivastava et al. (2010) suggested that a dose of 12.5 mg/kg-d (HED = 3 mg Co/kg-d) might be considered a hematological NOEL for Co, in our view this dose needs to be more appropriately classified as a LOEL because the reported effects of increased RBC, hemoglobin, and hematocrit are consistent with polycythemia (estimated blood Co concentrations of 810 µg/L, as reported in Table 2 and Figure 2).

As summarized in Figure 2, the lowest hematological LOEL is 320 µg/L from the Davis and Fields (1958) study as estimated by the biokinetic model (which assumed a response at 0.97 mg Co/kg-d and a 70 kg body weight for 22 days). Blood Co LOELs from other studies ranged from 600 to 2800 µg/L (Figure 2). The highest NOEL was 650 µg/L, as estimated from the Jaimet and Thode (1955) study with

TABLE 2. No Observed Effect Level and Effect Level Doses in Animals with the Corresponding Blood Concentrations

Reference	Exposed group	Response evaluated	Response Category	NOAEL (HED) (mg Co/kg-day)	LOAEL (HED) (mg Co/kg-day)	No response observed: estimated or reported blood Co levels (μg/L or ppb)	Response observed: estimated or reported blood Co levels (μg/L or ppb)
Roginski and Mertz, 1977	59 male Sprague Dawley rats	No change in hemoglobin levels with 10% lactal-albumin diet	Hematological	0.27 (0.065)		23	
Domingo et al., 1984	40 male Sprague Dawley rats	Polycythemia	Hematological		31.7 (7.6)		2800
Shrivastava et al., 2010	32 male Sprague Dawley rats	Polycythemia	Hematological		12.5 (3.0)		810
Mohiuddin et al., 1970	120 male guinea pigs	Tachypnea, pericardial effusion, thickened pericardium and myocardial degeneration	Cardiac		7.6 (2.2)		750
Domingo et al., 1984	40 male Sprague Dawley rats	Hypertrophy of the heart	Cardiac		31.7 (7.6)		2800
Morvai et al., 1993	32 male CFY rats	Myocytolysis and degeneration of myofibrilles	Cardiac		22.7 (5.5)		1800
Haga et al., 1996	56 male Sprague Dawley rats	Left ventricular hypertrophy and impaired left ventricular systolic and diastolic functions	Cardiac		8.4 (2.0)		750
Zak, 1968	18 male Albino rats	Decrease iodine uptake by the thyroid	Endocrine		1.8 (0.43)		150
Roginski and Mertz, 1977	36 male Sprague Dawley rats	No change in iodine uptake by the thyroid with 12% lactal-albumin diet	Endocrine	0.27 (0.065)		23	
Shrivastava et al., 1996	36 female mice	Histopathological changes in the thyroid	Endocrine		49.4 (6.9)		2100
Nation et al., 1983	18 male Sprague Dawley rats	Decrease rate of lever pressing relative to controls	Neurological	5 (1.2)	20 (4.8)	440[b] (430)	880[b] (1700)
Bourg et al., 1985	16 male Sprague Dawley rats	Increased behavioral reactivity to stress	Neurological		20 (4.8)		450[b] (1700)
Apostoli et al., 2012	21 rabbits	Optic system toxicity	Neurological				420[b]
Apostoli et al., 2012	21 rabbits	Auditory system toxicity	Neurological				781[b]
Nation et al., 1983	18 male Sprague Dawley rats	Testicular atrophy	Reproductive	5 (1.2)	20 (4.8)	440[b] (430)	880[b] (1700)
Domingo et al., 1984	40 male Sprague Dawley rats	Testicular atrophy	Reproductive		31.7 (7.6)		2800
Corrier et al., 1985	84 male Sprague Dawley rats	Testicular degeneration	Reproductive		20 (4.8)		1700
Mollenhaur et al., 1985	84 male Sprague Dawley rats	Testicular degeneration	Reproductive		20 (4.8)		1700
Pedigo et al., 1988	30 male and female CD1 mice	Adverse effects on spermatogenesis	Reproductive		23.0 (3.2)		1100
Anderson et al., 1992, 1993	10 male CD1 mice	Decreased testicular weight	Reproductive		43.4 (6)		2100
Pedigo and Vernon, 1993	$B^6C^3F^1$ mice	Decreased reproductive capacity of male mice	Reproductive		99.0 (14)		5000
Elbetieha et al., 2008	40 male Swiss mice	Decreased sperm count	Reproductive		11.6 (1.6)		590

The following dose adjustment factors (DAF) were utilized: 3.5 for guinea pigs, 4.16 for rats, andd 7.18 for mice. Animal doses were converted to Human Equivalent Doses (HED) before being used to estimate blood Co concentrations.

[b]Reported blood Co concentrations, estimated blood Co concentrations are in parathenses for oral ingestion studies.

*15% GI absorption was assumed. *All calculations used to estimate blood Co concentrations are provided in the online supplementary materials.

no hematological responses at 1.8 mg Co/kg-d. NOELs from other studies ranged from 1 to 200 μg/L.

ESTIMATED Co BLOOD CONCENTRATIONS ASSOCIATED WITH CARDIOVASCULAR EFFECTS

Cardiomyopathy has been established as a potential adverse effect in animals exposed to high concentrations of Co via oral and inhalation routes. The results from epidemiology studies, however, do not provide a clear picture of the doses at which adverse effects on the heart would be expected. In an evaluation of a Co-exposed cohort, Linna et al. (2004) reported an association between cumulative Co exposure and alterations in left ventricular filling and relaxation times, but the clinical significance of these changes remain unresolved. Although data are not presented in any detail, the aforementioned foundry worker study of Angerer et al. (1985) reported that no "signs of cardiomyopathy" were observed following rontgenology and electrocardiography in 40 individuals with Co blood concentrations from 4.9 to 47.9 μg/L (mean of 26 μg/L, reported as a NOAEL in Table 1 and Figure 3).

The beer-drinker cohorts that reported cardiomyopathic effects (Alexander, 1969, 1972; Morin et al., 1971) cannot be used to establish cardiac LOAELs because of the significant confounding effects of poor diet. Kesteloot et al. (1968) demonstrated that well-nourished beer drinkers experienced no cardiomyopathic effects at an estimated dose of 0.09 mg Co/kg-d (assuming a 70-kg body weight), which is equivalent to a NOAEL of 34 μg Co/L (Table 1 and Figure 3). However, malnourished beer drinkers with the identical estimated Co dose (0.09 mg Co/kg-d) suffered severe cardiomyopathic effects (Kesteloot et al., 1968). As shown in Figure 4, Kesteloot et al. (1968) and other studies (Sullivan et al., 1968; Morin et al., 1971; Alexander, 1972) indicated that adverse cardiac effects in malnourished alcoholics occurred at blood Co concentrations ranging from 15 to 34 μg/L. As discussed later, this finding was likely due to low circulating blood protein levels (the result of a very poor diet), which subsequently resulted in disproportionately high concentrations of free (non-protein-bound) Co in the blood and cardiac tissues.

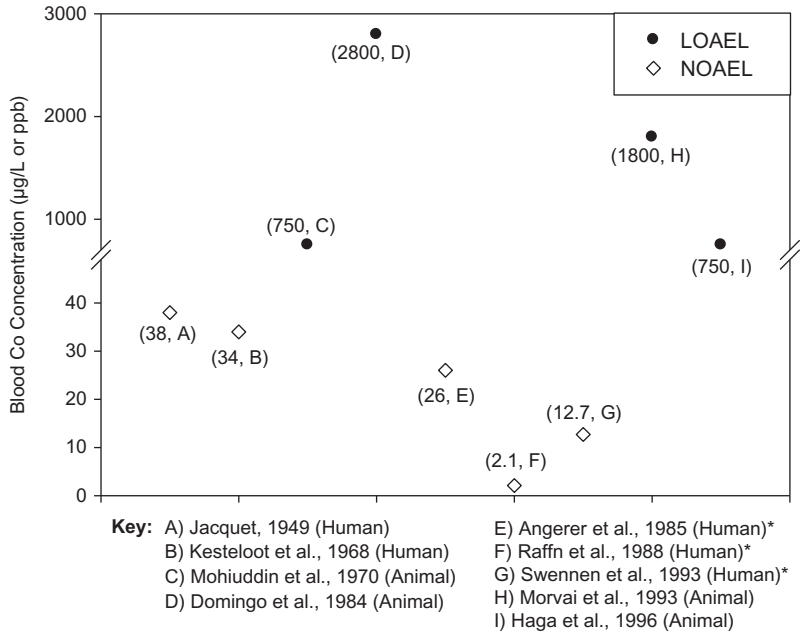

FIGURE 3. Reported or estimated blood Co NOAELs and LOAELs for cardiac effects. *Reported blood Co concentrations are denoted by an asterisk next to the study (*).*

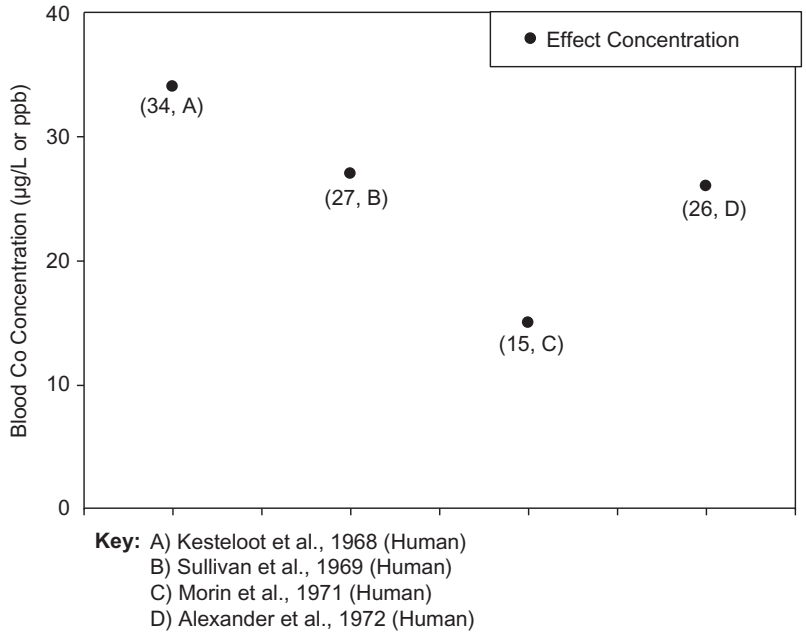

FIGURE 4. Reported or estimated blood Co concentrations associated with cardiac effects in susceptible subpopulations. *Reported blood Co concentrations are denoted by an asterisk next to the study (*).*

Jacquet (1949) noted no adverse cardiac effects were identified via electrocardiograms and arteriopiezography in patients treated for hypertension with 6.8–9.0 mg Co/d (0.097–0.13 mg Co/kg-d). The midpoint of the estimated blood concentrations (at 0.11 mg/kg-d) which produced no adverse effects (NOAEL) was 38 μg Co/L (Table 1 and Figure 3). In the aforementioned study of Swennen et al. (1993), in which workers were exposed to Co oxides, salts, and metals, no marked changes in serum concentrations of myocardial protein kinase were noted relative to a referent group, and hence 12.7 μg Co/L (mean value Friday, post shift) is considered to represent a cardiac NOAEL. Raffn et al. (1988) reported in the porcelain painter cohort that "the electrocardiograms showed few abnormalities, and no differences between the two groups were found" (the pulse rate was higher in the exposed group, but it was noted this was unrelated to blood Co levels). The mean blood concentration of Co of 2.1 μg/L is considered to be a cardiac NOAEL (Table 1 and Figure 3).

Regarding animal studies, Mohiuddin et al. (1970) reported cardiac effects in guinea pigs dosed with approximately 7.6 mg of Co/kg-d (HED = 2.2 mg Co/kg-d) in their diet or by oral gavage for 5 wk. Pericardial effusion was observed in approximately 50% of the Co-treated animals, and a substantial number of the animals reportedly developed tachypnea (LOAEL of 750 μg Co/L, Table 2 and Figure 3). Morvai et al. (1993) demonstrated that 3 wk of exposure to 22.7 mg Co/kg-d (HED = 5.5 mg Co/kg-day) in rats resulted in cardiac damage, presenting as incipient, multifocal myocytolysis, with degeneration of myofibriles (LOAEL of 1800 μg Co/L, Table 2 and Figure 3). Following longer term exposure (2–3 mo) of rats to 31.7 mg Co/kg-d (HED = 7.6 mg Co/kg-d), an increase in heart weight was found (Domingo et al., 1984) (LOAEL of 2800 μg Co/L, Table 2 and Figure 3). Haga et al. (1996) noted a temporal component to Co-induced cardiomyopathy; no marked effects were observed in rats exposed to 8.4 mg Co/kg-d for 16 wk, yet decreased left ventricular systolic and diastolic function were seen in rats exposed to this same dose for 24 wk (LOAEL of 750 μg Co/L, Table 2 and Figure 3) for 24 wk.

In summary, none of the studies that evaluated healthy (well-nourished) Co-exposed human cohorts reported adverse effects on the

cardiovascular system where blood concentrations in these cohorts ranged from 2.1 to 38 μg Co/L. Adverse cardiac effects were observed in animals exposed to Co doses of 7.6 mg Co/kg-d and higher. After applying the proper dosimetric adjustment factors, animal LOAEL ranged from 750 to 2800 μg/L (Table 2 and Figure 3).

ESTIMATED Co BLOOD CONCENTRATIONS ASSOCIATED WITH ENDOCRINE RESPONSES

Numerous case reports describe the development of endocrine effects such as goiters in individuals experiencing long-term (up to 7 mo) Co exposures of 0.45 to 10 mg/kg-d (Gross et al., 1955; Kriss et al., 1955; Little and Sunico, 1958; Paley et al., 1958; Chamberlain, 1961; Robey et al., 1956) (none of these case studies report blood Co data). Roche and Layrisse (1956) found that 2 wk of oral exposure to approximately 0.97 mg Co/kg-d in 12 adults resulted in inhibited uptake of radioactive iodine by the thyroid (estimated blood Co LOEL of 300 μg/L, shown in Table 1 and Figure 5). Jaimet and Thode (1955) evaluated 3 different endocrine responses including radioactive iodine uptake, radioiodine conversion ratio, and saliva protein-bound iodine activity ratio in children dosed at 0.45, 0.9, 1.8, or 2.7 mg Co/kg-d for 10 wk. For each endpoint, responses were observed at 2.7 mg/kg-d (LOEL), but not at 1.8 mg/kg-d (as shown in Figure 5, estimated blood Co NOEL and LOEL of 650 and 920 μg/L, respectively).

Bowie and Hurley (1975), reported no marked endocrine effects as measured by changes in serum T4 and TSH levels in 11 patients with measured blood Co concentrations ranging from 220 to 2100 μg/L (mean of approximately 600 μg/L shown as a NOEL in Table 1 and Figure 5. Swennen et al. (1993) reported a "slight" but statistically significant decrease (vs. an unexposed control group) in total (free and protein-bound) T3 concentrations, but no marked difference in T3 uptake, T4 levels, or TSH. Increased total T3 levels alone are not an indication of hypothyroidism (Mayo Clinic, 2012a), and, further, the mean total T3 levels in the exposed group (140.18 ng/dl) are well within the range of normal values (80–190 ng/dl; Mayo Clinic, 2012a). It is thus proposed that the mean blood Co value of 12.7 μg/L measured in the exposed

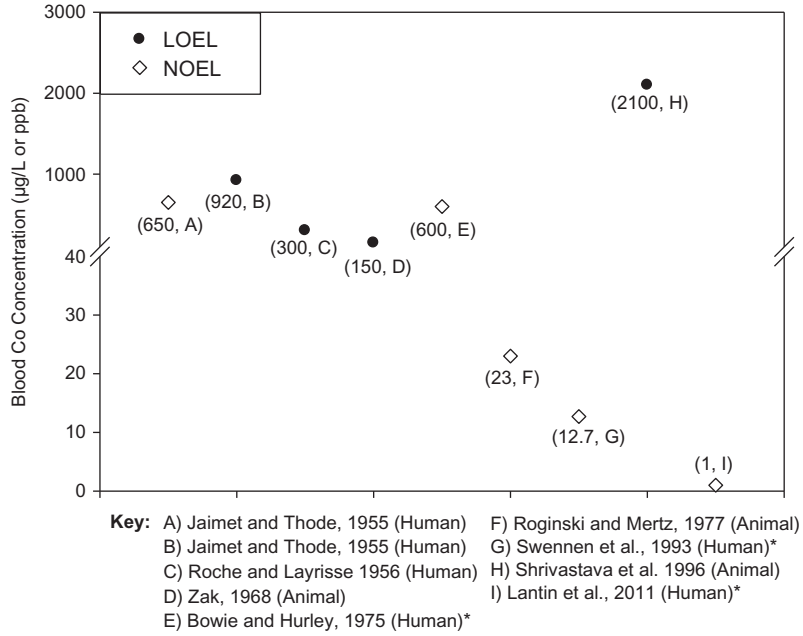

FIGURE 5. Reported or estimated blood Co NOELs and LOELs for endocrine effects. *Reported blood Co concentrations are denoted by an asterisk next to the study (*).*

cohort should be treated as an endocrine NOEL (Table 1 and Figure 5). Lantin et al. (2011) found no significant correlation between T4, TSH, or T3 levels with blood Co concentrations in a Co-exposed cohort, and therefore the median value of 1 μg Co/L is defined in that group as an endocrine NOEL (Table 1 and Figure 5).

The aforementioned study of Roginski and Mertz (1977) reported no alterations in iodine uptake by the thyroid of rats at an HED of 0.065 mg Co/kg-d (shown as a NOEL of 23 μg/L in Table 2 and Figure 5). Zak (1968) reported decreased iodine uptake in rats orally exposed to 1.8 mg Co/kg-d (HED = 0.43 mg Co/kg-d) for 60 days (LOEL of 150 μg/L). Shrivastava et al. (1996) reported histopathological changes in the thyroid gland of female mice exposed to 49.4 mg Co/kg-d (HED of 6.9 mg Co/kg-d) in drinking water for 15 to 45 d (LOEL of 2100 μg/L).

As summarized in Table 1 and Figure 5, blood Co LOELs range from 150-2100 μg/L. The blood Co NOELs ranged from 1-650 μg/L.

ESTIMATED Co BLOOD CONCENTRATIONS ASSOCIATED WITH NEUROLOGICAL EFFECTS

Regarding adverse neurological effects following Co exposure, most published cohort studies failed to establish a causal relationship. Jordan et al. (1990) reported memory deficits in 12 former hard metal workers exposed to tungsten carbide, Co, and other chemicals. However, because of the nonspecific nature of the reported effects and the presence of other workplace chemicals, they concluded that "a comparison of workers with a history of exposure to solvents without hard metals would permit a more complete evaluation of the neurophysiological sequelae of hard metal exposure." (p. 243).

Base on our review of the literature, the aforementioned analyses by Duckham and Lee (1976) and Bowie and Hurley (1975) are the only published papers that reported blood Co measurements and neurological evaluations in a human cohort. Duckham and Lee (1976) evaluated auditory acuity using the Rinne and Weber tests and polyneuropathy in 4 patients with blood Co concentrations ranging from 640 to 1220 μg/L (mean of approximately 900 μg/L) after 12 wk of Co therapy, and noted that "none of our patients suffered any clinically detectable eighth-nerve damage. Similarly, no patient developed peripheral neuropathy" (Duckham and Lee, 1976, p. 291). An addendum to the paper noted that one patient (A.T.) eventually developed "slight" high-tone deafness after another 40 wk of additional Co therapy. For the purposes of this analysis, the mean of all the values collected during the first 12 wk of therapy (900 μg/L) was considered as a blood concentration that produced no marked effects on the neurological system (e.g., a NOAEL) (Table 1 and Figure 6).

Similarly, Bowie and Hurley (1975) reported that in 12 dialysis patients with measured blood Co concentrations ranging from 220 to 2100 μg/L (after 8 wk of Co therapy), none of the patients developed tinnitus or deafness, but three patients (T.B., J.W., M.D.) demonstrated a "bilateral rise in threshold of at least 20 decibels in the range over 6000 Hz and over 5decibels in the lower ranges down to 2.50 Hz" (p. 312). One month after treatment ended, two of these patients' audiograms returned to normal (J.W., M.D.), while the other (T.B.) was unavailable for repeat examination. It is interesting to note that these patients had 3 of the 4 highest (J.W., 2100 μg Co/L; M.D., 600 μg/L; T.B., 560 μg/L) blood Co levels measured at the 8-wk time point. For the purposes of this analysis, the mean of the blood Co concentrations measured in these three patients (1087 μg/L) was considered as a neurological LOAEL (Table 1 and Figure 6), and the mean of the blood Co concentrations in the other subjects (approximately 440 μg/L) as a neurological NOAEL (Table 1 and Figure 6). Meecham and Humphrey (1991) noted a reversible vision failure and progressive bilateral deafness with tinnitus in a 48-yr-old man with a history of occupational exposure to Co powder. Three months after quitting his job, his total blood Co concentration was still quite elevated at 234 μg/L; according to the biokinetic model,

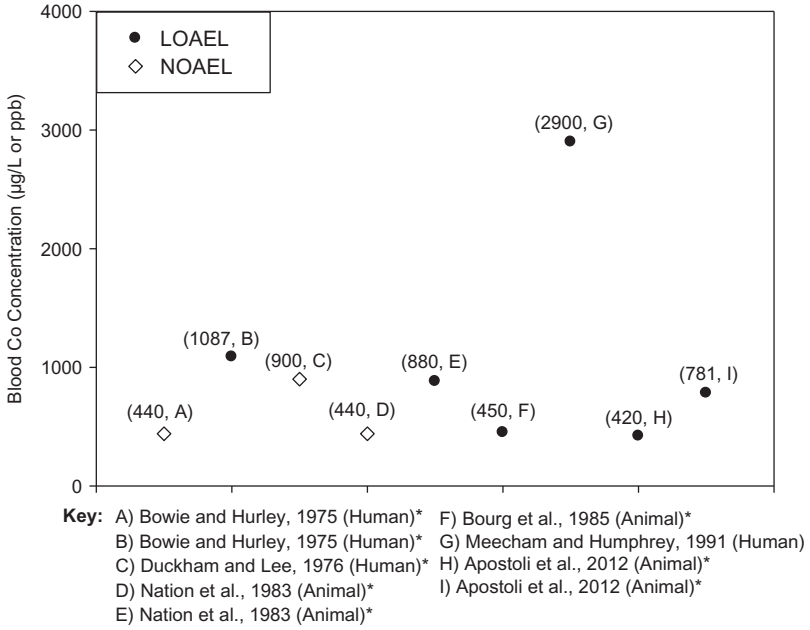

FIGURE 6. Reported or estimated blood Co NOAELs and LOAELs for neurological effects. *Reported blood Co concentrations are denoted by an asterisk next to the study (*).*

his blood Co concentration at the end of his employment would have been approximately 2900 µg/L (reported as a LOAEL in Table 1 and Figure 6). His vision and hearing returned to normal approximately 1 yr after quitting his job (Meecham and Humphrey, 1991).

Other reports of neurological evaluations in individuals on Co therapy are presented in Table 3 (Gardner, 1953; Licht et al., 1972; Schirrmacher, 1967; Schleisner, 1956). None of these cases reported blood Co concentrations, and, as indicated in the table, in almost every case the patient had some form of renal dysfunction. It is therefore not possible to accurately estimate blood Co concentrations for these individuals. Similar to the findings of Duckham and Lee (1976) and Bowie and Hurley (1975), the hearing and vision losses often resolved once Co therapy was discontinued.

TABLE 3. Case reports of Co-associated neurological effects

Reference	Effect	Dose	Duration	Preexisting health effects
Licht et al., 1972	Impaired vision due to optic atrophy in a 32 yr old M undergoing Co therapy	113 mg Co/day	15 wks	Pancytopenia and hypercellular bone marrow
Schleisner, 1956	Hearing loss in a 52 yr old F anephric patient undergoing Co therapy	50 mg Co/day	40 wks	Renal disease
Schirrmacher, 1967	Reversible bilateral nerve deafness and hearing loss in a 35 yr old F anephric patient undergoing Co therapy	45.4 mg Co/day	6 months	Renal disease
Gardner, 1953	Reversible high frequency hearing loss in an anephric patient undergoing Co therapy, sex and age not specified	36.3 mg Co/day	16 wks	Renal disease
Gardner, 1953	Reversible feet paresthesias in an anephric patient undergoing Co therapy, sex and age not specified	182 mg Co/day	6 wks	Renal disease
Gardner, 1953	Tinnitus in four anephric patients undergoing Co therapy, sex and age not specified	22.7 to 90.8 mg Co/day	4–16 wks	Renal disease

While there are some animal studies (e.g. Krasovskii and Fridliand, 1971; Singh and Junnarkar, 1991) that reported certain behavioral responses following dosing with Co, such as food aversion or decreased lever pressing rates, none of the findings can be extrapolated to any of the neurological responses observed in humans (reversible hearing and vision disturbances) exposed to very high levels of Co. One exception is the study of Apostoli et al. (2012), in which it was recently reported that rabbits with blood Co concentrations of approximately 420 ug/L and 781 ug/L suffered from optic and auditory system toxicity (both values are listed as LOAELs in Table 2 and Figure 6). These blood Co concentrations and effects are consistent with the hearing and visual disturbances reported in humans with highly elevated blood Co concentrations (Bowie and Hurley, 1975; Meecham and Humphrey, 1991). Interestingly, neurological effects were observed in rabbits at blood levels below those that caused no such effects in humans and therefore suggests that rabbits may be more sensitive to Co.

The animal studies of Nation et al. (1983) and Bourg et al. (1985) warrant some discussion because, as shown in Table 2, these studies reported blood Co concentrations and behavioral responses as a function of oral Co dose. Nation et al. (1983) in rats exposed to 5 and 20 mg Co/kg-d for 69 d showed no marked change in reactivity to pre-aversive or aversive stimuli during the tests for conditioned suppression; however, a decrease in operant level press rates was noted at 20 mg/kg-d. Based on Figure 3 in Nation et al. (1983), it was estimated that the approximate average wet weight blood Co concentration was 0.4 μg/g in the 5 mg/kg-d group ($n = 6$) and 0.9 μg/g in the group exposed to 20 mg/kg-d ($n = 6$). Assuming a blood density of 1.1 g/cm^3, the blood concentrations were approximately 440 μg/L (NOAEL) and 880 μg/L (LOAEL) in the 5- and 20-mg Co/kg-d groups, respectively (Table 2 and Figure 6). Bourg et al. (1985) found that rats orally exposed to 20 mg Co/kg-d for 57 d displayed enhanced emotional reactivity to stress, and reported a mean blood Co concentration of 0.408 μg Co/g tissue (LOAEL of 450 μg Co/L, as shown in Table 2 and Figure 6) in these animals.

ESTIMATED Co BLOOD CONCENTRATIONS ASSOCIATED WITH REPRODUCTIVE EFFECTS

No studies were identified that reported adverse reproductive effects in humans following Co exposure by any route. However, in the study by Holly (1955b), it was reported that "no toxic manifestations following cobalt administration to 78 pregnant women. All children resulting from these pregnancies were found to be normal" (p. 1352). These mothers had been dosed with 100 mg Co/day (Holly, 1955a; Holly 1955b).

Animals exposed to high Co doses have experienced certain adverse reproductive effects. Elbetieha et al. (2008) examined the effects of Co on fertility in 40 adult male Swiss mice exposed to 200, 400, or 800 ppm $CoCl_2$ (11.6 mg Co/kg-d, 21.3 mg Co/kg-d, or 42.2 mg Co/kg-d) via drinking water for 12 wk. Exposure to the two highest doses resulted in numerous adverse reproductive effects, including: reduced fertility, reduced number of implantation sites, reduced number of viable fetuses, and reduced testicular weight. Decreased sperm count was also observed at all doses and, therefore, we adopted the lowest dose of 11.6 mg/kg-d (HED = 1.6 mg Co/kg-d) as the LOAEL (blood Co concentration of 590 μg/L, as shown in Table 2 and Figure 7).

Testicular degeneration was reported in Sprague-Dawley rats exposed to approximately 20 mg Co/kg-d (HED = 4.8 mg Co/kg-d) for 70 d (Corrier et al., 1985; Mollenhauer et al., 1985). Pedigo et al. (1988) reported decreased spermatogenesis at 23 mg Co/kg-d (HED = 3.2 mg Co/kg-d) in CD-1 mice exposed via drinking water for 7–11 wk (LOAEL of 1100 μg/L). Male mice exposed to the same dose (99 mg Co/kg-d) have also been reported to have decreased reproductive capacity (Pedigo and Vernon, 1993). In addition, Nation et al. (1983) demonstrated testicular atrophy in rats exposed

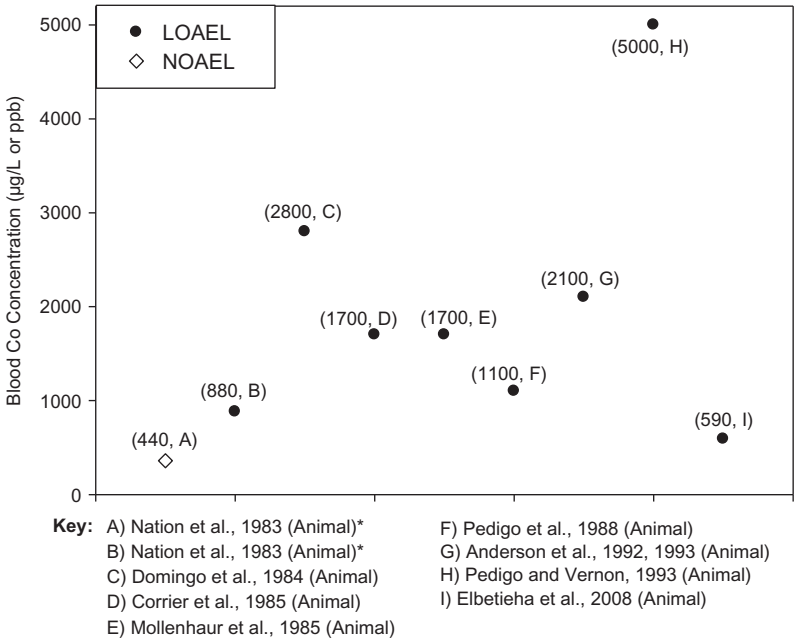

FIGURE 7. Reported or estimated blood Co NOAELs and LOAELs for reproductive effects. *Reported blood Co concentrations are denoted by an asterisk next to the study (*).*

to 20 mg Co/kg-d; no atrophy was observed at 5 mg Co/kg-d. Results from other animal studies also reported Co induced reproductive toxicity, although at much higher doses (Domingo et al., 1984; Anderson et al., 1992;1993). It is important to note that the reproductive studies employed doses that approached the LD50 and/or elicited measures of frank toxicity (reduced body weight, etc.) in the exposed animals. As summarized in Table 2 and Figure 7, Co blood LOAEL for reproductive effects in animals ranged from 450 to 5000 µg/L, and a NOAEL of 440 µg/L was reported in Nation et al. (1983).

MISCELLANEOUS STUDIES

Many studies were considered for this analysis; however some were not included due to irregular dosing regimens or questionable relevance of the animal model. For example, Pimentel-Malaussena et al., (1958) reported decreased iodine uptake by the thyroid in some patients dosed with Co; however, each of the patients received multiple doses for various durations, so it is not possible to pinpoint a representative dose and duration of exposure for the cohort.

Polycythemia has been reported in chickens dosed with approximately 7.1 mg Co/kg-d (Diaz et al., 1994), while Huck and Clawson (1976) reported no changes in hematological parameters in pigs with elevated blood Co concentrations (~10 µg/L). In a study by Sandusky et al. (1981), Co-related effects were evaluated in dogs maintained on a normal diet or a protein and thiamine deficient diet. However, the relevance of these test species (chickens, pigs, dogs) to humans is unclear and as a result, we have not utilized them to identify NOELs or LOELs in this study.

DISCUSSION

The purpose of this study was to identify dose-response relationships between blood Co concentrations and adverse health effects. The average or peak blood concentration is typically the best metric for assessing exposure to exogenous agents, so this was the focus of this analysis. Approximately 30 animal and human studies were evaluated,

and in each study the maximum blood Co concentration not associated with any response or adverse effects (NOEL or NOAEL, respectively) and/or the lowest blood Co concentration at which a response or effect occurred (LOEL or LOAEL, respectively) was identified. Endocrine, cardiovascular, hematological, reproductive, and neurological endpoints were evaluated because effects in each of these organ systems have been observed in animals or humans exposed to Co above certain doses.

The Biokinetic Model

In this paper, we frequently converted oral Co doses to estimated blood Co concentrations, using a biokinetic model (Unice et al., 2012). To evaluate the accuracy of the model, we recently completed a volunteer study involving the ingestion of Co. Specifically, Tvermoes et al. (2012) conducted a screening study in which 4 adult human male volunteers ingested approximately 400 μg Co/d of a Co supplement (Mineralife) for 14 d (blood samples were drawn and analyzed for Co pre-dosing and at several days during the dosing period). Blood Co concentrations pre-dosing were less than 0.5 μg Co/L, while mean concentrations during dosing ranged from approximately 2 to 4 μg Co/L. The mean measured concentration at 14 d was within 5% of the biokinetic model predictions, if 15–35% of the oral supplement is assumed to be absorbed into systemic circulation (in a 70-kg male). These findings indicated that (1) the Co biokinetic model accurately predicted blood Co concentrations in this group of individuals; and (2) Co ingestion at doses considered "safe" (400 μg/d) will yield approximately 2–4 μg Co/L in the blood of an adult human male.

Many of the no-effect and effect levels summarized in Figures 2–7 were derived using oral Co dose-response results as input values to the Co biokinetic model; however, the doses were generally higher than the 400-μg Co/d (approximately 0.005 mg Co/kg-d) dose used in the volunteer study. In those instances in which measured blood Co concentrations were reported in oral dosing studies involving subjects with healthy, functioning kidneys, the fit with the modeled estimates seemed to vary as a function of dose.

Specifically, as shown in Table 2, the measured blood Co concentration in rats exposed to 5 mg Co/kg-d (Nation et al., 1983) was approximately 440 μg Co/L, which is consistent with the biokinetic model estimate of 430 μg Co/L. However, at the higher dose of 20 mg Co/kg-d, which was employed in both Nation et al. (1983) and Bourg et al. (1985), there was less concordance: the measured blood values were approximately 450 μg Co/L (Bourg et al., 1985) and 880 μg Co/L (Nation et al., 1983), and the modeled value was 1700 μg Co/L. The reason for the larger discrepancy at the higher dose is likely due to our assumption that a consistent percentage (15%) of the oral dose is absorbed regardless of the magnitude of exposure. It is known that percent GIT absorption of Co decreases as the dose increases over some (as yet undefined) threshold (Barceloux, 1999; Reuber et al., 1994; Taylor, 1962), and therefore it is possible that percent GIT absorption was less than 15% at 20 mg/kg-d. Consequently, blood Co concentrations for some of the other high dose animal studies (e.g., Domingo et al., 1984; Shrivastava et al., 1996; Anderson et al., 1993) may also be overestimated.

The biokinetic model far underpredicts blood Co levels in individuals with a nonfunctioning renal system. As shown in Table 1, the estimated blood Co concentrations from the patients in Duckham and Lee (1975) and Bowie and Hurley (1975) were up to almost 1000 μg Co/L lower than the measured values. This finding is not unexpected, because renal failure patients on Co therapy (for anemia) often accumulate high Co blood levels. Curtis et al. (1976) measured Co blood levels in two hemodialysis patients and one normal subject receiving 50 mg $CoCl_2 \bullet 6H_2O$/d for 2 wk and found that blood Co values in the hemodialysis patients after 2 wk of dosing were significantly higher than those measured in the normal subject: approximately 400 and 800 μg Co/L, versus approximately 100 μg Co/L in the normal subject. It is also important to note

that Fe deficiency has been shown to increase Co absorption in both animals and humans (Barceloux, 1999; Reuber et al., 1994; Sorbie et al., 1971; Valberg et al., 1969), and, indeed, recent findings suggest that Co and Fe may share a common intestinal uptake mechanism that is upregulated with anemia and associated lower oxygen content in blood (Karovic et al., 2007). Accordingly, patients who are either anemic and anephric or both, as in Duckham and Lee, (1976) and Bowie and Hurley (1975), would be expected to display higher blood Co concentrations than those predicted by the biokinetic model. This model assumes normal kidney clearance of Co and a relatively low GIT uptake of 15%.

Healthy Versus Susceptible Populations

Kesteloot et al. (1968) first observed that significant malnourishment in the beer drinker cohorts was associated with enhanced susceptiblity to Co-induced adverse cardiac effects. In healthy individuals, approximately 90% of blood Co is bound to albumin and other proteins, while the remainder exists as "free" (unbound) Co ion (Jansen et al., 1996; Onkelinx, 1976). We have estimated that 10% of the Co is "free" in healthy individuals at blood Co concentrations up to at least 1000 μg/L. The increased susceptibility in the malnourished individuals was likely due to low circulating levels of blood proteins, which would have resulted in higher concentrations of free Co ion than would be found in a healthy individual with the same total blood Co levels. Various degrees of exposures or physiological states that push this equilibrium towards increased amounts of free Co ion may also increase the likelihood that an adverse response to Co might occur.

Evaluating the Strength of the Data for Each Organ System

Hematological Responses Increased hemoglobin levels and RBC counts were the desired effects associated with oral Co therapy in the 1950s and 1960s. As shown in Figure 2, the blood Co NOELs for hematological responses in various human and animal studies ranged up to 650 μg/L as estimated from Jaimet and Thode (1955). The blood Co LOELs were generally higher, up to 2800 μg/L, with the exception of the 320 μg/L value from Davis and Fields (1958). Jaimet and Thode (1955) reported no marked increases in hemoglobin levels in children at 1.8 mg Co/kg-d for 10 wk, while Davis and Fields (1958) noted polycythemia in adult humans at only 0.97 mg/kg-d for 3 wk or less. This discrepancy might be due to differences in sensitivity between children and adults regarding polycythemic responses, although there are no known reports describing such differences. Alternatively, it is possible that, contrary to the conclusions of Davis and Fields (1958), the numerical increases in RBC and percent hemoglobin levels (11 and 21%, respectively) simply were not clinically or statistically sigificant and that these results are not indicative of Co-related effect. Interestingly, Davis and Fields (1958) reported that the increased RBC levels returned to baseline conditions within days after Co dosing ended. Given that newly formed RBC typically exist for approximately 90 d, this behavior is fairly anomalous, and we are unaware of any other studies involving polycythemia, Co-induced or otherwise, in which this type of transient elevation was observed, thus further suggesting that perhaps polycythemia did not occur in this study.

Overall, the data indicate that blood Co concentrations of approximately 300 μg/L or less have not been associated with hematological responses, while blood Co concentrations of approximately 600 μg/L and higher have consistently been associated with polycythemia, increased hemoglobin content, and similar responses. It is important to note that the hematological responses in human studies were actually efficacious responses to therapeutic doses of Co, and hence, we concluded that these responses do not warrant the same degree of concern as irreversible Co-induced cardiomyopathy or adverse reproductive effects. It is also worth

noting that polycythemia is considered to be one of the most sensitive responses following Co exposure. For example, the Agency for Toxic Substances and Disease Registry (ATSDR, 2004) chose to use Davis and Fields (1958) as the basis of its estimated "minimal risk level," a value typically based on the biological effects or responses that occur at the lowest dose. Similarly, Finley et al. (2012) recently published a methodology for deriving a proposed Co "reference dose" (a dose intended to be protective against all adverse effects in the general population) based on hematological (and endocrine) responses observed in human and animal studies. These findings are consistent with the present analysis: hematological responses occurred at blood Co concentrations as low as 300–600 μg/L, while adverse cardiac, neurological, and reproductive effects were usually only observed at blood Co concentrations of approximately 800 μg/L and higher.

Endocrine Responses Endocrine responses following Co exposure were occasionally observed as unwanted side effects in a small fraction of anemic patients undergoing Co therapy. In the present analysis, thyroid dysfunction was considered to be a sensitive indicator of endocrine effects in both human and animal studies. As presented in Table 1 and Figure 5, blood Co concentrations up to 650 μg/L were not associated with endocrine responses in humans. The human endocrine LOEL of 300 μg Co/L estimated from Roche and Layrisse (1956) (based on reduced thyroid uptake of iodine) is lower than the NOELs of 650 μg/L from Jaimet and Thode (1955), as well as the human NOEL of 600 μg/L from Bowie and Hurley (1975). The reasons for this inconsistency are unclear. The endocrine responses were (1) typically reversible upon cessation of Co exposure; (2) not necessarily indicative of long-term adverse effects; and (3) one of the more sensitive endpoints of Co exposure. With respect to the latter point, changes in thyroid function as observed in Jaimet and Thode (1955) were considered as a basis for a Co reference dose (Finley et al., 2012), and the U.S. EPA also recently proposed an endocrine-based Co reference dose using the results of Roche and Layrisse (1956).

Cardiac Effects Many of the cardiovascular studies involved animals exposed to a single Co dose level; effects were observed in all of these studies because of the massive doses administered (there was no observed NOAEL in the animal studies). In humans, it is clear that Co-induced adverse effects are dependent on the health and nutritional status of the exposed individual. None of the studies that evaluated healthy (well-nourished) Co-exposed cohorts (including the beer drinkers) reported adverse effects on the cardiovascular system at blood Co concentrations of 2.1–38 μg/L (Table 1 and Figure 3), but malnourished individuals (the beer drinker cohorts with poor diets) experienced severe cardiomyopathy at approximately the same blood Co concentrations (15–34 μg/L, as shown in Figure 4).

As the work of Kesteloot et al. (1968) first indicated, the enhanced susceptibility to the Co-induced cardiomyopathic effects noted in these studies is likely due to a relatively high concentration of free Co ions in blood and tissues that occurs as a result of reduced blood albumin and protein binding capacity. At the blood Co concentrations estimated for the beer drinker cohorts (generally between 10 and 34 μg/L), the malnourished individuals probably had a higher concentration of bioavailable (unbound) Co than did their otherwise healthy (well-nourished) counterparts. Clearly, the influence of free versus bound Co in the blood, and the implications for potential health effects, is an area that warrants more research.

As seen in Figure 3, the available data for the cardiac endpoint are somewhat unique, in that there is a relatively large margin between the maximal NOAEL value (38 μg/L) and the minimal LOAEL value (750 μg/L). There is little to no such margin between the NOAEL and the LOAEL for all other endpoints. Consistent with the U.S. EPA and analyses of the Co toxicology literature, and the Co reference dose proposed in Finley et al. (2012), it is postulated that Co-induced cardiomyopathy is a relatively insensitive effect, and may not occur at all in healthy individuals. While it would be helpful to

have more data on healthy cohorts with blood Co concentrations in the 200–500 μg/L range, it is proposed that cardiac effects will not occur at blood Co concentrations below those that are associated with hematological and endocrine responses, and would therefore only occur in healthy persons at blood Co concentrations of 300 μg/L or higher.

Neurological Effects There is considerable overlap between the neurological NOAELs and LOAELs (Figure 6) with most values clustered between 400 and 1000 μg Co/L. Bourg et al. (1985) reported a neurological LOAEL of 450 μg/L from an animal study showing increased behavioral reactivity to stress, which would appear to conflict with the neurological NOAELs of 440 and 900 μg/L from Bowie and Hurley (1975) and Duckham and Lee (1976) demonstrating hearing loss and polyneuropathy. Given that the latter two studies involve humans, and the questionable relevance of "stress"-related endpoints measured in animals, the findings of Bowie and Hurley (1975) and Duckham and Lee (1976) are considered to be more predictive of Co-related adverse neurological effects in humans.

Table 3 presents several case reports of hearing and vision disturbances in anemic individuals undergoing Co therapy. It is noteworthy that in almost every case except Licht et al. (1972), the patient had some form of renal dysfunction. For the cases in Table 3, the biokinetic model predicted that blood Co concentrations would have ranged from 110 to 950 μg/L. However, as seen in the comparisons of "measured versus modeled" blood Co estimates from Duckham and Lee (1976) and Bowie and Hurley (1975) (Table 1), the biokinetic model consistently underestimated actual blood Co concentrations in dialysis patients by at least four- to fivefold, suggesting that blood Co concentrations in the patients in Table 3 were all likely to have exceeded 500 μg/L, and may have been higher than 1000 μg/L in some cases. Further, it is known that dialysis may reduce blood protein levels, which indicates that dialysis patients on Co treatment showed not only high concentrations of blood Co because of poor clearance, but also that a relatively large fraction of that Co was not bound to blood proteins and was therefore available to distribute into tissues and potentially produce adverse effects. This reasoning likely explains why adverse neurological effects were observed in these individuals at oral Co doses similar to those that did not exert any marked effects in patient cohorts with normal kidneys (Davis and Fields, 1958; Jaimet and Thode, 1955; Roche and Layrisse, 1956). Licht et al. (1972) is the only case of neurological effects occurring in a Co-dosed patient with healthy kidneys; however, the prescribed dose for that patient (113 mg Co/d) was extraordinarily high, and the estimated blood Co concentration (biokinetic model) for that individual is 590 μg/L.

In short, adverse neurological effects in Co-dosed humans have largely been reported only in patients with kidney disease, which suggests that even high-dose oral Co therapy simply does not result in a blood Co concentrations sufficient to produce neurological effects unless the individual is unable to efficiently clear Co (free and unbound) from the bloodstream. Our findings suggest that, as with cardiac effects, adverse neurological effects have typically not been observed except at blood Co concentrations of approximately 800 μg/L and higher.

Interestingly, in many of the cases that reported sight and/or hearing loss, the symptoms partially or completely resolved following cessation of Co exposure (Bowie and Hurley, 1975; Gardner, 1953; Meecham and Humphrey, 1991; Schirrmacher, 1967). As recently reported in Rubin (2012), reversible sensory effects occur in nearly 25% of patients with a hypothyroid disease such as lymphocytic thryoiditis, pituitary injury or disease, or severe iodine deficiency, and the most common clinical features associated with hypothyroid neuropathy are a symmetric sensory disturbance in the feet and hands with tingling and painful dysthesias in addition to hearing and vision loss (Anand et al., 1989; Yamamoto et al., 1983). These are the same neurological symptoms described in renal failure patients receiving high-Co-dose therapy.

The pathogenesis of hypothyroid neurological deficits is not completely understood, but the temporary loss of vision in hypothyroid individuals is thought to occur as a result of pituitary hyperplasia, resulting from a decreased negative feedback from thyroid hormone (Khawaja et al., 2006). Interestingly, some neurological effects occurring as a result of exposure to Co are consistent with the neurological disturbances identified in persons with hypothyroidism. For example, hypothyroidism-induced dementia is by and large understood to be a treatable form of dementia with thyroid replacement (Rubin, 2012). The onset and duration of these neurological symptoms also appear to be generally correlated with the onset and duration of hypothyroidism (Nemni et al., 1987). Again, this finding is consistent with the simultaneous cessation of adverse endocrine and neurological effects in patients on high Co-dose therapy. Of the neurological cases reported in Tables 1 and 3, some demonstrated the presence of adverse endocrine effects including hypothyroidism (Schirrmacher, 1967), while others did not (Bowie and Hurley, 1975) as evidenced by absence of decreased serum T4 or TSH. In at least some cases the neurological symptoms appear to be associated with excessive Co exposure as a result of hypothyroidism, and not as a result of direct action on the central or peripheral nervous system.

Reproductive Effects The only evaluation of reproductive effects in Co-exposed humans is that of Holly (1955b), wherein it was reported that "no toxic manifestations" were observed in pregnant women dosed with 100 mg Co/day. Several animal studies have reported a variety of adverse reproductive effects following exposure to high oral Co doses (Figure 7), but it is difficult to extrapolate these results to humans because all of the studies employed doses that approached the LD50 and/or produced indicators of general toxicity in the exposed animals. This endpoint is likely to be the least sensitive of all the organ systems we evaluated.

Cancer endpoints Although this analysis focuses on effect-levels for noncancer systemic effects, it is worth pointing out that the potential carcinogenic effects of Co have been evaluated by others. For example, the National Toxicology Program conducted a 2-year Co inhalation study in which tumor incidence was measured in various tissues (NTP 1998). Animals were exposed to 0.3, 1 or 3 mg/m3 CoSO4 heptahydrate 6 h/day, 5 d/week for 105 weeks. The authors reported there was clear evidence of increased lung tumors in male and female mice and female rats. In addition, female rats had an increased incidence of pheochromocytoma (tumor) of the adrenal medulla. The incidence of follicular cell hyperplasia of the thyroid gland was reported to be moderately increased in all exposed groups of male mice, but no dose-response relationship was noted. The incidence of neoplasms in the liver, pancreas, cardiovascular system, nervous system, thyroid gland and thymus of exposed animals was not increased relative to controls. There are no animal studies indicating that Co is an oral carcinogen. A similar weight of evidence exists for humans: certain forms of Co appear to be able to induce respiratory tract tumors following elevated inhalation exposures in the workplace, but there is no evidence to suggest that any other route of exposure is associated with a cancer risk. The EPA has stated "Human studies are inconclusive regarding inhalation exposure to cobalt and cancer, and the one available oral study did not report a correlation between cobalt in the drinking water and cancer deaths" (EPA, 2007, ¶1). To date, the EPA has not classified Co as a human carcinogen.

Blood Co Concentrations and Health Effects in Metal Hip Implant Patients

It has been known for decades that Co blood concentrations are typically elevated in individuals with Co-containing hip implants (Coleman et al., 1973; Jacobs et al., 1996; MacDonald et al., 2003). The presence of Co in blood occurs because of a combination of wear and corrosion (Catelas et al., 2006; Hart et al., 2010). A vast majority of the published blood Co values for metal implant patients are between 0.1 and 10 μg/L (Antoniou et al., 2008; Brodner et al., 2003; Engh et al., 2009;

Vendittoli et al., 2007; MacDonald et al., 2003; Walter et al., 2008). Hence, blood Co concentrations in most patients are similar to concentrations associated with off-the-shelf Co supplement ingestion, and far below the blood Co LOAELs and even most NOAELs identified in this analysis.

There are case reports that describe high blood Co concentrations in hip implant patients, and these cases often report the types of adverse health effects described in this paper. For example, hypothyroidism, peripheral neuropathy, and cardiomyopathy were noted in a failed hip implant patient with Co blood concentrations peaking at 625 μg/L (Oldenburg et al., 2009). Ikeda et al., (2010) described neuropathy, hearing loss, and hypothyroidism in a patient with Co blood concentrations exceeding 400 μg/L as a result of a deformed CoCr head; however, the patient's symptoms improved after surgery. Decreased hearing, optic atrophy, and feet numbness were also reported in a 53-yr-old man 2 yr after a metal implant was used to replace a shattered ceramic device, where his serum Co concentration at the time of revision was 398 μg/L, but fell to 36 μg/L at 8 wk after revision, and was less than 1 μg/L at a 6-mo follow up appointment (Steens et al., 2006). After revision surgery, the numbness in his feet disappeared, his hearing returned, and his vision improved (Steens et al., 2006). Rizzetti et al. (2009) and Pelclova et al. (2012) noted peak blood Co concentrations of 549 and 506 μg/L, respectively, in patients with failed CoCr hip implants. Visual and auditory disturbances in conjunction with hypothyroidism were reported in Rizzetti et al. (2009), while Pelclova et al. (2012) reported paresthesias and hearing loss.

In many of the abovementioned cases, the blood Co concentrations sometimes exceeded the LOAEL values for hypothyroidism (about 300 μg/L and higher) and therefore thyroid dysfunction in such cases is consistent with what we would expect based on our analyses. However, reports of vision and hearing disturbances as well as numbness are not consistent with the available clinical data reporting blood Co concentrations. Specifically, Bowie and Hurley (1975) reported no hearing loss in nine dialysis patients with a mean blood Co concentration of 440 μg/L, while Duckham and Lee (1976) observed no peripheral neuropathy or clinically significant hearing loss in four anephric patients with a mean blood Co concentration of 900 μg/L (individual values were as high as 1220 μg/L). To our knowledge, hearing loss as a result of Co exposure has only been reported in three hemodialysis patients with a mean peak Co serum level of 1087 μg/L (Bowie and Hurley 1975). Likewise, reports of cardiomyopathy at these blood Co concentrations are not consistent with the clinical literature involving high dose Co therapy. The reasons for this discrepancy are unclear but if the responses were truly Co-related then it is likely the individuals had one or more risk factors for susceptibility. Indeed, at least some of the case reports described above involved patients with renal failure, a condition that can result in decreased binding of Co to serum albumin. This is an area that warrants further investigation.

There are also some instances in which similar symptoms have been reported in hip implant patients at far lower blood Co concentrations, far below the LOAELs identified in this analysis, but the role of Co or any other etiological factor is less clear. In some of these cases, there is simply a lack of complete discussion of the patient's medical history. For example, Tower (2010) reported cognitive decline as evidenced by hearing loss and dyspnea in one patient with a Co serum concentration of 23 μg/L, and dyspnea, tinnitus/hearing loss, cognitive decline, and optic atrophy in a second patient (this patient was actually Dr. Tower himself) with a serum Co concentration of 122 μg/L. However, no clinical data pre or post surgery or post implant removal were available in the paper, and the reporting of symptoms was anecdotal. Similarly, Mao et al. (2011) noted a serum Co concentration of 15 μg/L in a patient 3 yr post implant and indicated that the patient had experienced a decrease in cognitive function "although this was not quantified." In other cases, there appear to be numerous complicating factors that make

it difficult to ascribe the reported effects to Co exposure. In Mao et al. (2011), an implant patient with a serum Co concentration of 24 μg/L reportedly displayed neurological symptoms, including cognitive decline, memory difficulties, and depression, at 5 yr post implant, but it was also noted that these conditions had been present since a cerebrovascular incident ("consistent with a stroke") 7 mo prior. Machado et al. (2012) found a variety of cardiac problems in a patient diagnosed 6 yr after receiving a Co-containing implant, but the patient was also 75 yr old, morbidly obese, and hypercholesterolemic. At present there is sufficient evidence to indicate that the reported symptoms in these cases are related to Co exposure.

Several recent review articles discuss potential Co-associated systemic toxicity from hip prostheses (Campbell and Estey 2012; Polyzois et al. 2012; Gill et al. 2012). However, these reports do not provide a thorough systematic assessment of the risk associated with increased internal Co exposure. For example, Polyzois et al. (2012) offered a hypothesis that Co nanoparticles formed due to wear debris might increase cytotoxicity. The authors cited in vitro tests to support their hypothesis although it is unclear whether these results would be relevant to systemic effects nor at what blood concentrations one would be concerned. In the Gill et al (2012) paper, nano-particle issues and the possible risk of systemic toxicity were also discussed, and the authors concluded that additional testing would be required to characterize any human health risks due to implants. Lastly, Campbell and Estey (2012) suggest that "cobalt and chromium concentrations should not be used in isolation when assessing a patient with a MoM hip prosthesis" (p. 7). However, our analysis indicates that blood Co concentrations are a valuable predictor of adverse effects. Although we acknowledge that the presence of both Co and Cr in the blood, concurrently, could theoretically interact in an antagonistic or synergistic manner, we would not expect either to occur because the blood Co and Cr concentrations are generally too low. In those few cases where the concentrations in the synovial fluid of both cobalt and chromium are in the thousands of μg/L, the possibility of frank cellular toxicity (e.g., local effects at the joint tissue) cannot be ruled out at this time. Overall, the information in these three recent reviews does not seem to be in conflict with this analysis.

Various Proposed Guidelines for Acceptable Blood Co Concentrations

Some groups have proposed specific blood Co concentrations that they suggest could be considered benchmarks for protecting against adverse health effects or other endpoints. In all cases, these blood concentrations are far below the blood Co LOAEL (and even NOAEL) identified in this paper. The American Conference of Industrial Hygienists (ACGIH) established a biological exposure index (BEI) of 1 μg/L in blood as a benchmark for all inorganic forms of Co (ACGIH, 2001). This value is the blood concentration estimated to result from inhalational Co exposure at the threshold limit value (TLV) 8-h time-weighted average (TWA) of 0.02 mg/m^3. However, the BEI is based primarily on protection against respiratory effects, which is the primary route of exposure in the workplace; therefore, this guideline is not necessarily relevant to systemic, extrapulmonary effects such as those addressed in this analysis.

Similarly, a guidance value of 7 μg Co/L in blood was suggested by the UK Medicines and Healthcare products Regulatory Agency (MHRA) to identify metal hip implant patients who may require closer surveillance because they are likely to be experiencing excessive wear (MHRA, 2012). This value is not health based, but rather is an upper bound of a distribution of blood Co values measured in a group of hip implant patients (Sampson and Hart, 2011). The objective of their guideline was to use blood Co levels to identify patients who are experiencing excessive wear. Likewise, the Mayo Clinic website suggests that Co-related effects might occur at blood Co concentrations of 5 μg/L and greater if "cobalt is ingested" (Mayo Clinic, 2012b). However, no information is presented regarding the derivation of this value. All of these proposed values are below the estimated human blood Co

concentrations associated with ingestion of Co at 600 μg/d (5.7 μg/L) and/or 1400 μg/d (13 μg/L). As mentioned earlier, these are daily doses suggested by the United Kingdom Expert Group on Vitamins and Minerals (EGVM) and the European Food Safety Authority (EFSA) (respectively) to be without any apparent risk of adverse health effects.

Uncertainties in Our Analysis

There are some sources of uncertainty in the blood Co effect levels described here that merit discussion. In those cases in which the biokinetic model was used to estimate blood Co concentration in animal and human oral dosing studies, it was assumed that only 15% of the oral dose was systemically absorbed. Since it is known that GIT absorption rates in humans actually range up to 35% and higher, often in women (which can approach 70% absorption) the blood Co concentrations were probably underestimated in some studies. Therefore, some of the no-effect and effect level values are likely biased low. In addition, the biokinetic model was partially developed from adult human kinetic data; while it is proposed that the model is accurate for adult humans, there may be some uncertainty in applying the model to children. Further, the database on females may have been insufficient to determine whether a different GIT absorption rate needs to be used for different categories of women (e.g., of child bearing years, those who exercise aggressively).

In some cases, Co doses were considered as LOELs, even though the authors reported the findings as NOELs. Jaimet and Thode (1955) concluded that endocrine responses were not observed at the highest dose (2.7 mg Co/kg-d) employed in their study, even though two of the participants had to be withdrawn because of the development of significant endocrine responses. Shrivastava et al. (2010) concluded that an oral dose of 12.5 mg Co/kg-d in rats could be considered a hematological NOEL, but at that dose the exposed group experienced a statistically significant increase in RBC, hemoglobin, and hematocrit. Conversely, some doses were defined as NOELs even though the authors reported that responses or effects had been observed. Both Swennen et al. (1993) and Raffn et al. (1988) noted decreases in one or more hematological parameters such as RBC levels in humans occupationally exposed to Co; however, it is likely that these responses were not Co-related as Co induces the opposite response. Swennen et al. (1993) also found "a slight interference with thyroid metabolism" based on reduced T3 levels, but it was concluded that the balance of the other, more relevant endocrine endpoints including T3 uptake, T4, and TSH levels (none of which were significantly different from control values) indicated that the workers did not experience a Co-related endocrine response. Overall, though, contrary interpretations and/or exclusion of these studies does not significantly influence the range of blood Co no-effect and effect level values derived for any given health endpoint.

The exposure durations were highly variable from study to study, and some of the no-effect values were derived from studies with fairly long exposures. The 26 μg/L NOAEL for hematological and cardiac effects from Angerer et al. (1985) involved workplace Co exposures of up to 36 yr; the cardiac NOAELs of 34–38 μg/L derived from both Jacquet et al. (1949) and Kesteloot et al. (1968) also followed months to years of human Co exposure. Limited evidence suggests that at higher exposures, there may be a temporal aspect of Co toxicity. Specifically, Haga et al. (1996) found no marked cardiac effects in rats exposed to 8.4 mg Co/kg-d (estimated blood Co concentration of 750 μg/L) for 16 wk, yet decreased left ventricular systolic and diastolic function were observed in rats exposed to this same dose for 24 wk. At least for the endocrine endpoint, the U.S. EPA indicated that if reduced iodide uptake does not occur following short-term (14- to 90-d) studies, then chronic exposure under the same conditions is unlikely to produce hypothyroidism: "Chronic exposure will have no greater effect than that resulting from short term exposure, because if the precursor event of inhibition of iodide uptake does not occur, then there will be no changes in thyroid

function in the short or long term. Prolonged exposure may actually have less effect because of the capacity of the pituitary-thyroid system to compensate for iodide deficiency by increasing iodide uptake" (U.S. EPA, 2012, Section I.A.3). Clearly, however, this issue would benefit from more investigation.

Other uncertainties that might warrant additional research include (1) the degree to which blood protein binding influences the relative amount of free versus protein-bound Co that might make an individual more susceptible to the effects of Co; (2) a better understanding of the molecular mechanisms by which Co initiates various disease processes and whether or not certain adverse effects such as endocrine and neurological effects are related; and (3) refinement of the biokinetic model to ensure accurate application to non-adult humans.

CONCLUSIONS

This analysis provides the first comprehensive assessment of blood Co concentrations associated with specific health outcomes. Individuals who may have elevated blood Co concentrations include patients on Co therapy, subjects who ingest Co supplements for real or perceived health benefits, those who ingest foods high in Co content, workers handling Co-containing materials, individuals exposed to Co-contaminated environmental media, and patients with Co-containing metal hip implants. Our analysis found that biological responses and adverse effects in humans were not observed below measured or estimated blood Co concentrations of 300 μg/L, but were consistently observed at approximately 700–800 μg/L and higher. These findings may be used to evaluate the potential risk to Co-exposed individuals or cohorts with elevated blood Co concentrations, and further refinement of the dose-response relationships established in this paper might find applications in other areas.*

NOTE

*All calculations used to estimate blood Co concentrations are provided in the online supplementary materials.

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